

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau



attach
to file
#4

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification⁵: C12N 15/54, 9/12, 1/21, 5/10, C07K 15/28, C12Q 1/48, A61K 37/52, 48/00	A2	(11) International Publication Number: WO 94/17189 (43) International Publication Date: 4 August 1994 (04.08.94)
(21) International Application Number: PCT/US94/00795 (22) International Filing Date: 21 January 1994 (21.01.94) (30) Priority Data: 08/008,001 21 January 1993 (21.01.93) US (71) Applicant: THE SALK INSTITUTE FOR BIOLOGICAL STUDIES [US/US]; 10010 North Torrey Pines Road, La Jolla, CA 92037 (US). (72) Inventor: HOEKSTRA, Merl. F.; 10321 216th Street, S.E., Shobomish, WA 98290 (US). (74) Agent: NOLAND, Greta, E.; Marshall, O'Toole, Gerstein, Murray & Borun, 6300 Sears Tower, 233 South Wacker Drive, Chicago, IL 60606 (US).		(81) Designated States: CA, JP, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Published <i>Without international search report and to be republished upon receipt of that report.</i>
(54) Title: PROTEIN KINASES (57) Abstract Protein kinase mutant and wild-type genes encoding polypeptides of the class heretofore designated "casein kinase I" and useful in screening compositions which may effect DNA double-strand break repair activity are disclosed. Also disclosed are methods using the polynucleotides in cell-proliferative disorders.		

09/34/505

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	GB	United Kingdom	MR	Mauritius
AU	Australia	GE	Georgia	MW	Malawi
BB	Barbados	GN	Guinea	NE	Niger
BE	Belgium	GR	Greece	NL	Netherlands
BF	Burkina Faso	HU	Hungary	NO	Norway
BG	Bulgaria	IE	Ireland	NZ	New Zealand
BJ	Benin	IT	Italy	PL	Poland
BR	Brazil	JP	Japan	PT	Portugal
BY	Belarus	KE	Kenya	RO	Romania
CA	Canada	KG	Kyrgyzstan	RU	Russian Federation
CF	Central African Republic	KP	Democratic People's Republic of Korea	SD	Sudan
CG	Congo	KR	Republic of Korea	SE	Sweden
CH	Switzerland	KZ	Kazakhstan	SI	Slovenia
CI	Côte d'Ivoire	LI	Liechtenstein	SK	Slovakia
CM	Cameroon	LK	Sri Lanka	SN	Senegal
CN	China	LU	Luxembourg	TD	Chad
CS	Czechoslovakia	LV	Latvia	TG	Togo
CZ	Czech Republic	MC	Monaco	TJ	Tajikistan
DE	Germany	MD	Republic of Moldova	TT	Trinidad and Tobago
DK	Denmark	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	US	United States of America
FI	Finland	MN	Mongolia	UZ	Uzbekistan
FR	France			VN	Viet Nam
GA	Gabon				

09/341505

- 1 -

PROTEIN KINASES

09/341505 12 JUL 1999

This application is a Continuation-in-Part of U.S. Application Serial No. 08/008,001, filed January 21, 1993, which is a Continuation-in-Part of U.S. Application Serial No. 728,783, filed July 3, 1991.

5

FIELD OF THE INVENTION

The present invention relates to novel polynucleotides encoding polypeptides which correspond to the class of protein kinase isolates heretofore referred to as casein kinase I and which possess protein kinase and/or DNA recombination/repair promoting functional capabilities.

10

BACKGROUND OF THE INVENTION**Protein Kinases**

The protein kinases comprise an exceptionally large family of eukaryotic proteins which mediate the responses of cells to external stimuli and are related by amino acid sequence homology within the so-called "catalytic domain" of the enzymes. To date, in excess of 100 unique members of the protein kinase family from a wide variety of eukaryotic organisms have been described and characterized at the amino acid sequence level. See, e.g., Hanks, *et al.* (*Science*, 241:42-52, 1988) which presents a sequence alignment of 65 protein kinase catalytic domains which range in size from about 250 to 300 amino acids and Hanks, *et al.* (*Methods in Enzymol.*, 200:38-62, 1991) presenting a catalytic domain sequence alignment for 117 distinct protein kinase family members including a variety of vertebrate, invertebrate, higher plant and yeast species enzymes. The location of the catalytic domain within a protein kinase is not fixed. In most single subunit enzymes, the domain is near the carboxy terminus of the polypeptide while in multimeric protein kinases the catalytic domain takes up almost the entirety of the subunit polypeptide.

25

Protein kinases are generally classified into a protein-serine/threonine subfamily or a protein-tyrosine subfamily on the basis of phosphorylation substrate specificity. Among the many classes of enzymes within

-2-

the protein-serine/threonine kinase subfamily are two distinct classes which have been designated casein kinase I and casein kinase II based on the order of their elution from DEAE-cellulose. The casein kinases are distinguished from other protein kinases by their ability to phosphorylate serine or threonine residues within acidic recognition sequences such as found in casein. Tuazon, *et al.*, (*Adv. in Second Messenger and Phosphoprotein Res.*, 23:123-164, 1991) presents a review of over 200 publications related to casein kinase I and II, addressing the physicochemical characterization, recognition sequences, substrate specificity and effects on metabolic regulation for these two classes of enzymes. Casein kinase II is active as a heterotetramer and the complete amino acid sequences of human, rat, *Drosophila* and yeast species catalytic regions have been determined. Despite the fact that partially purified casein kinase I preparations have been obtained from cell nuclei, cytoplasm, and cell membranes of various plant and animal species, prior to the present invention, nothing was known concerning the primary structure of its enzymatically active monomeric subunit.

As of the time of the present invention, therefore, there existed a significant need in the art for information concerning the primary structure (amino acid sequence) of protein-serine/threonine kinase enzymes of the casein kinase I class. Such information, provided in the form of DNA sequences encoding one or more of these kinases (from which primary structures could be deduced), would allow for the large scale production of kinases by recombinant techniques as well as for determination of the distribution and function of these enzymes, the structural distinctions between membrane-bound and non-membranous forms, the potential ligand-receptor interactions in which these kinases interact, and the identification of agents capable of modulating ligand-receptor binding, kinase, and other activities.

DNA Recombination And Repair

Chromosomes experience single-stranded or double-stranded breaks as a result of energy-rich radiation, chemical agents, as well as spontaneous breaks occurring during replication among others. Although genes present in the chromosomes undergo continuous damage, repair, exchange, transposition, and

-3-

splicing, certain enzymes protect or restore the specific base sequences of the chromosome.

The repair of DNA damage is a complex process that involves the coordination of a large number of gene products. This complexity is in part dependent upon both the form of DNA damage and cell cycle progression. For example, in response to ultraviolet (UV) irradiation, cells can employ photoreactivation or excision repair functions to correct genetic lesions. The repair of strand breaks, such as those created by X-rays, can proceed through recombinational mechanisms. For many forms of DNA damage, the cell is induced to arrest in the G2 phase of the cell cycle. During this G2 arrest, lesions are repaired to ensure chromosomal integrity prior to mitotic segregation.

Since the transfer of genetic information from generation to generation is dependent on the integrity of DNA, it is important to identify those gene products which affect or regulate genetic recombination and repair. Through the use of organisms with specific genetic mutations, the normal functional gene can be obtained, molecularly cloned, and the gene products studied.

In eukaryotes such as *Saccharomyces cerevisiae*, genetic studies have defined repair-deficient mutants which have allowed the identification of more than 30 radiation-sensitive (*RAD*) mutants (Haynes, *et al.*, in *Molecular Biology of the Yeast Saccharomyces*, pp. 371, 1981; J. Game in *Yeast Genetics: Fundamental and Applied Aspects*, pp. 109, 1983). These mutants can be grouped into three classes depending upon their sensitivities. These classes broadly define excision-repair, error-prone repair, and recombinational-repair functions. The molecular characterization of yeast *RAD* genes has increased the understanding of the enzymatic machinery involved in excision repair, as well as the arrest of cell division by DNA damage.

The understanding of *RAD* genes and their expression products has become increasingly important as research continues to develop more effective therapeutic compositions. Often these new compositions appear quite effective against a particular disease condition, such as certain tumors, but prove to be too toxic for *in vivo* therapy in an animal having the disease. Indeed, these compositions can actually increase the likelihood of mutagenesis.

-4-

Most agents that are mutagenic or carcinogenic are in themselves unreactive, but are broken down to reactive intermediates *in vivo*. It is these reactive intermediates which interact with DNA to produce a mutation. This event is thought to be the initial step in chemical carcinogenesis. Mutations in a large number of genes affect the cellular response to agents that damage DNA. In all likelihood, many of these mutated genes encode enzymes that participate in DNA repair systems. Consequently, when the repair system is compromised, the cells become extremely sensitive to toxic agents. Although the DNA may revert to normal when DNA repair mechanisms operate successfully, the failure of such mechanisms can result in a transformed tumor cell which continues to proliferate.

Although there are currently available tests to determine the toxicity or mutagenicity of chemical agents and compositions, there are limitations in both laboratory screening procedures and animal toxicity tests. These limitations include extrapolating laboratory data from animals to humans. There is often a large measure of uncertainty when attempting to correlate the results obtained in laboratory animals with effects in human subjects. In most cases, doses of the test drug have been used in the animal which are too high to be safely administered to humans. In addition, some types of toxicity can be detected if the drug is administered in a particular species, yet may be missed if the experiment is not done in the correct animal species. Moreover, many currently available laboratory tests are incapable of detecting certain types of toxic manifestations which occur in man.

Phenotypic complementation, as a way of identifying homologous normal functional genes, is widely used. For example, the human homologue of the yeast cell cycle control gene, *cdc 2*, was cloned by expressing a human cDNA library in *Schizosaccharomyces pombe* and selecting those clones which could complement a mutation in the yeast *cdc 2* gene (Lee, *et al.*, *Nature*, 327:31, 1987). A mammalian gene capable of reverting the heat shock sensitivity of the *RAS2^{val19}* gene of yeast, has also been cloned by using complementation (Colicelli, *et al.*, *Proc. Nat'l. Acad. Sci. USA*, 86:3599, 1989). A rat brain cDNA library was used to clone a mammalian cDNA that can complement the loss of

-5-

growth control associated with the activated RAS2 gene in yeast. The gene, DPD (dunce-like phosphodiesterase), encodes a high-affinity CAMP phosphodiesterase.

In summary, limitations and uncertainties of existing laboratory tests fail to provide an accurate method of examining the effects of a composition on DNA integrity. In view of this, a considerable need exists for screening methodologies which are inexpensive, rapid, and contain the relevant gene from the animal which is to be treated with the composition. Such methods provide a direct assay to determine if a composition interferes with the DNA repair system of a cell.

10

SUMMARY OF THE INVENTION

In one of its aspects, the present invention provides purified and isolated polynucleotides (e.g., DNA sequences and RNA transcripts thereof) encoding eukaryotic protein kinases of the casein kinase I class herein designated as "*HRR25*-like" proteins and characterized by greater than 35% amino acid sequence homology with the prototypical yeast enzyme *HRR25* through the protein kinase catalytic domain thereof. Polynucleotides provided by the invention include RNAs, mRNAs and DNAs, including antisense forms thereof. Preferred DNA sequences of the invention include genomic and cDNA sequences as well as wholly or partially chemically synthesized DNA sequences and biological replicas thereof. Specifically illustrating the invention are *Saccharomyces cerevisiae* DNAs including those encoding *HRR25* and *NUF1*, *Schizosaccharomyces pombe* DNAs including those encoding *Hhp1* + and *Hhp2* +, and human DNAs including those encoding *CKI α 1Hu*, *CKI α 2Hu*, *CKI α 3Hu*, *CKI γ 1Hu*, *CKI γ 2Hu*, and *CKI δ Hu*. Also provided are autonomously replicating recombinant constructions such as plasmid and viral DNA vectors incorporating such sequences and especially vectors wherein DNA encoding an *HRR25*-like casein kinase I protein is linked to an endogenous or exogenous expression control DNA sequence.

According to another aspect of the invention, host cells, especially unicellular host cells such as procaryotic and eukaryotic cells, are stably transformed with DNA sequences of the invention in a manner allowing the

-6-

desired polypeptides to be expressed therein. Host cells expressing such *HRR25*-like products can serve a variety of useful purposes. To the extent that the expressed products are "displayed" on host cell surfaces, the cells may constitute a valuable immunogen for the development of antibody substances

5 specifically immunoreactive therewith.

Host cells of the invention are conspicuously useful in methods for the large scale production of *HRR25*-like proteins wherein the cells are grown in a suitable culture medium and the desired polypeptide products are isolated from the cells or from the medium in which the cells are grown.

10 Also comprehended by the present invention are antibody substances (e.g., monoclonal and polyclonal antibodies, single chain antibodies, chimeric antibodies, CDR-grafted antibodies and the like) and other binding proteins which are specific for *HRR25*-like proteins (i.e., non-reactive with protein kinase molecules which are not related by at least 35% homology with

15 *HRR25* through the protein kinase catalytic domain). Antibody substances can be developed using isolated natural or recombinant *HRR25*-like proteins or cells expressing such products on their surfaces. The antibody substances are useful, in turn, for purifying recombinant and naturally occurring *HRR25*-like polypeptides and identifying cells producing such polypeptides on their surfaces.

20 The antibody substances and other binding proteins are also manifestly useful in modulating (i.e., blocking, inhibiting, or stimulating) ligand-receptor binding reactions involving *HRR25*-like proteins. Anti idiotypic antibodies specific for anti-*HRR25*-like antibody substances are also contemplated. Assays for the detection and quantification of *HRR25*-like proteins on cell surfaces and in fluids

25 such as serum and cytoplasmic fractions may involve a single antibody substance or multiple antibody substances in a "sandwich" assay format.

Recombinant *HRR25*-like protein products obtained according to the invention have been observed to display a number of properties which are unique among the eukaryotic protein kinases. As one example, the *HRR25* protein

30 possesses both protein-tyrosine kinase and protein-serine/threonine kinase activities. Moreover, *HRR25* operates to promote repair of DNA strand breaks

-7-

at a specific nucleotide sequence and is the only protein kinase known to have such recombination/repair promoting activity.

The DNA sequence information for yeast and mammalian (including human) species *HRR25*-like proteins which is provided by the present invention makes possible the identification and isolation of DNAs encoding other *HRR25*-like proteins by such well-known techniques as DNA/DNA hybridization and polymerase chain reaction (PCR) cloning.

Recombinant *HRR25*-like proteins and host cells expressing the same are useful in screening methods designed to examine the effects of various compositions on DNA break repair and protein kinase activities of the proteins. Protein kinase inhibitory effects may be assessed by well-known screening procedures such as described in Hidaka, *et al.* (*Methods in Enzymology*, 201:328-339, 1991).

BRIEF DESCRIPTION OF THE DRAWING

Further aspects and advantages of the present invention will be apparent upon consideration of the following detailed description of presently preferred embodiments thereof, reference being made to the drawing wherein:

Figure 1 (A) presents an alignment of the predicted amino acid sequence of *HRR25* with the catalytic domains of the yeast CDC28, yeast KSS1 and human RAF1 protein kinases. Figure 1(B) shows a schematic representation of the structure of *HRR25*, and

Figure 2 presents an alignment of the predicted amino acid sequences of *HRR25* with the sequences of three other *Saccharomyces cerevisiae* *HRR25*-like proteins (YCK1/CKI2, YCK2/CKI1, and NUF1), two *HRR25*-like proteins (*Hhp1* + and *Hhp2* +) from *Schizosaccharomyces pombe* and three putative isoforms (CKI α 1Hu, CKI α 2Hu, and CKI α 3Hu) of a human *HRR25*-like protein.

DETAILED DESCRIPTION OF THE INVENTION

In one of its aspects, the present invention relates to a DNA encoding a recombination/repair promoting polypeptide which can be used in an assay system to examine the effects of various compositions on DNA integrity.

5 These functional sequences, which can be characterized by their ability to promote restoration of DNA strand breaks, permit the screening of compositions to determine whether a particular composition has an effect on the restoration of such repair activity. The invention also provides a DNA sequence encoding a polypeptide which promotes normal mitotic recombination, but is defective in
10 protein kinase activity and essentially unable to repair DNA strand breaks. This defective DNA sequence is highly useful for identifying other DNA sequences which encode proteins with functional protein kinase activity. In addition, the present invention relates to the polypeptide encoded by the defective DNA sequence, as well as the polypeptide encoded by the functional wild-type DNA.

15 In order to identify a DNA sequence encoding a polypeptide with protein kinase activity, a method is provided whereby a DNA library is screened for nucleotide sequences capable of restoring DNA strand break repair in a mutant lacking such activity. A method is further provided for identifying a composition which affects the activity of a mammalian polypeptide having protein kinase
20 activity, wherein the polypeptide is capable of restoring DNA double-strand break repair activity in a mutant lacking such activity.

In general, the defective protein kinase can be characterized by its ability to promote normal mitotic recombination, while being essentially unable to repair DNA double-strand break including that which occurs at the cleavage
25 site:

↓
CAACAG
GTTGTC
↑

30 The DNA double-strand breaks which the defective protein kinase is essentially unable to repair can be induced by various means, including endonucleases, x-rays, or radiomimetic agents including alkylating agents. Preferred endonucleases

-9-

are those which recognize the same nucleotide cleavage site as endonuclease *HO*. Radiomimetic alkylating agents having methylmethane sulfonate activity are preferred. Those of skill in the art will be able to identify other agents which induce the appropriate DNA strand breaks without undue experimentation.

5 The present invention specifically discloses mutants sensitive to continuous expression of the DNA double-strand endonuclease *HO*, which codes for a 65 kDa site-specific endonuclease that initiates mating type interconversion (Kostriken, *et al.*, *Cold Spring Harbor Symp. Quant. Biol.*, 49:89, 1984). These mutants are important to understanding the functions involved in recognizing and
10 repairing damaged chromosomes. This invention also discloses a yeast wild-type DNA recombination and repair gene called *HRR25* (*HO* and/or radiation repair). Homozygous mutant strains, *hrr25-1*, are sensitive to methylmethane sulfonate and X-rays, but not UV irradiation. The wild-type gene encodes a novel protein kinase, homologous to other serine/threonine kinases, which appears critical in
15 activation of DNA repair functions by phosphorylation.

 The *HRR25* kinase is important for normal cell growth, nuclear segregation, DNA repair and meiosis, and deletion of *HRR25* results in cell cycle defects. These phenotypes, coupled with the sequence similarities between the
20 *HRR25* kinase and the *Raf/c-mos* protein kinase subgroup suggest that *HRR25* might play a similar role in *S. cerevisiae* growth and development. The defects in DNA strand break repair and the aberrant growth properties revealed by mutations in *HRR25* kinase, expands the role that protein kinases may play and places *HRR25* in a functional category of proteins associated with DNA metabolism.

25 The development of specific DNA sequences encoding protein kinase polypeptides of the invention can be accomplished using a variety of techniques. For example, methods which can be employed include (1) isolation of a double-stranded DNA sequence from the genomic DNA of the eukaryote; (2) chemical synthesis of a DNA sequence to provide the necessary codons for the
30 polypeptide of interest; and (3) *in vitro* synthesis of a double stranded DNA sequence by reverse transcription of mRNA isolated from a eukaryotic donor cell.

-10-

In the latter case, a double stranded DNA complement of mRNA is eventually formed which is generally referred to as cDNA.

The novel DNA sequences of the invention include all sequences useful in providing for expression in prokaryotic or eukaryotic host cells of polypeptides which exhibit the functional characteristics of the novel protein kinase of the invention. These DNA sequences comprise: (a) the DNA sequences as set forth in SEQ. I.D. No. 1 or their complementary strands; (b) DNA sequences which encode an amino acid sequence with at least about 35% homology in the protein kinase domain with the amino acid sequences encoded by the DNA sequences defined in (a) or fragments thereof; and (c) DNA sequences defined in (a) and (b) above. Specifically embraced in (b) are genomic DNA sequences which encode allelic variant forms. Part (c) specifically embraces the manufacture of DNA sequences which encode fragments of the protein kinase and analogs of the protein kinase wherein the DNA sequences thereof may incorporate codons which facilitate translation of mRNA. Also included in part (c) are DNA sequences which are degenerate as a result of the genetic code.

The term "conservative variation" as used herein denotes the replacement of an amino acid residue by another, biologically similar residue. Examples of conservative variations include the substitution of one hydrophobic residue such as isoleucine, valine, leucine or methionine for another, or the substitution of one polar residue for another, such as the substitution of arginine for lysine, glutamic for aspartic acids, or glutamine for asparagine, and the like.

With the DNA sequences of the invention in hand, it is a routine matter to prepare, subclone, and express smaller DNA fragments from this or a corresponding DNA sequences. The term "polypeptide" denotes any sequence of amino acids having the characteristic activity of the mutant or wild-type protein kinase of the invention, wherein the sequence of amino acids is encoded by all or part of the DNA sequences of the invention.

The polypeptide resulting from expression of the DNA sequence of the invention can be further characterized as being free from association with other eukaryotic polypeptides or other contaminants which might otherwise be associated with the protein kinase in its natural cellular environment.

-11-

Isolation and purification of microbially expressed polypeptides provided by the invention may be by conventional means including, preparative chromatographic separations and immunological separations involving monoclonal and/or polyclonal antibody preparation.

5 In general, recombinant expression vectors useful in the present invention contain a promotor sequence which facilitates the efficient transcription of the inserted eukaryotic genetic sequence. The expression vector typically contains an origin of replication, a promoter, and a terminator, as well as specific genes which are capable of providing phenotypic selection of the transformed
10 cells. The transformed hosts can be grown in fermentors and cultured according to techniques known in the art to achieve optimal cell growth. The polypeptides of the invention can then be isolated from the growth medium, cellular lysates, or cellular membrane fractions.

The DNA sequences of the present invention can be expressed *in vivo* in either prokaryotes or eukaryotes. Methods of expressing DNA sequences containing eukaryotic coding sequences in prokaryotes are well known in the art. Biologically functional viral and plasmid DNA vectors used to incorporate DNA
15 sequences of the invention, for expression and replication in the host cell are well known in the art. For example, DNA can be inserted in yeast using appropriate vectors and introducing the product into the host cells. Various shuttle vectors for the expression of foreign genes in yeast have been reported (Heinemann, *et al.*, *Nature*, 340:205, 1989; Rose, *et al.*, *Gene*, 60:237, 1987). Those of skill in the art will know of appropriate techniques for obtaining gene expression in both
20 prokaryotes and eukaryotes, or can readily ascertain such techniques, without undue experimentation.
25

Hosts include microbial, yeast, insect and mammalian host organisms. Thus, the term "host" is meant to include not only prokaryotes, but also such eukaryotes such as yeast, filamentous fungi, as well as plant and animal cells which can replicate and express an intron-free DNA sequence of the
30 invention. The term also includes any progeny of the subject cell. It is understood that not all progeny are identical to the parental cell since there may

-12-

be mutations that occur at replication. However, such progeny are included when the terms above are used.

Transformation with recombinant DNA may be carried out by conventional techniques well known to those skilled in the art. Where the host is prokaryotic, such as *E. coli*, competent cells which are capable of DNA uptake can be prepared from cells harvested after exponential growth phase and subsequently treated by the CaCl_2 method using procedures well known in the art. Alternatively, MgCl_2 or RbCl could be used in the reaction. Transformation can also be performed after forming a protoplast of the host cell.

Where the host is a eukaryote, various methods of DNA transfer can be used. These include transfection of DNA by calcium phosphate-precipitates, conventional mechanical procedures such as microinjection, insertion of a plasmid encased in liposomes, spheroplast electroporation, salt mediated transformation of unicellular organisms or the use of virus vectors.

Analysis of eukaryotic DNA has been greatly simplified since eukaryotic DNA can be cloned in prokaryotes using vectors well known in the art. Such cloned sequences can be obtained easily in large amounts and can be altered *in vivo* by bacterial genetic techniques and *in vitro* by specific enzyme modifications. To determine the effects of these experimentally induced changes on the function and expression of eukaryotic genes, the rearranged sequences must be taken out of the bacteria in which they were cloned and reintroduced into a eukaryotic organism. Since there are still many functions in eukaryotic cells which are absent in prokaryotes, (e.g., localization of ATP-generating systems to mitochondria, association of DNA with histones, mitosis and meiosis, and differentiation of cells), the genetic control of such functions must be assessed in a eukaryotic environment. Cloning genes from other eukaryotes in yeast has been useful for analyzing the cloned eukaryotic genes as well as other yeast genes. A number of different yeast vectors have been constructed for this purpose. All vectors replicate in *E. coli*, which is important for amplification of the vector DNA. All vectors contain markers, e.g., LEU 2, HIS 3, URA 3, that can be selected easily in yeast. In addition, these vectors also carry antibiotic resistance markers for use in *E. coli*.

-13-

Many strategies for cloning human homologues of known yeast genes are known in the art. These include, but are not limited to: 1) low stringency hybridization to detect shared nucleotide sequences; 2) antibody screening of expression libraries to detect shared structural features; and 3) 5 complementation of mutants to detect genes with similar functions.

For purposes of the present invention, protein kinases which are homologous can be identified by structural as well as functional similarity. Structural similarity can be determined, for example, by assessing amino acid homology or by screening with antibody, especially a monoclonal antibody, which 10 recognizes a unique epitope present on the protein kinases of the invention. When amino acid homology is used as criteria to establish structural similarity, those amino acid sequences which have homology of at least about 35% in the protein kinase domain with the prototypical *HRR25* protein are considered to uniquely characterize polypeptides.

15 Conserved regions of amino acid residues in *HRR25* can be used to identify *HRR25*-like genes from other species. Conserved regions which can be used as probes for identification and isolation of *HRR25*-like genes (homologues) include the nucleotides encoding amino acid sequences GPSLED (amino acids 86 to 91 in SEQ ID NO: 2), RDIKPDNFL (amino acids 127 to 135 20 in SEQ ID NO: 2), HIPYRE (amino acids 164 to 169 in SEQ ID NO: 2), and SVN (amino acids 181 to 183 in SEQ ID NO: 2), for example. These conserved motifs can be used, for example, to develop nucleotide primers to detect other *HRR25*-like genes by methods well known to those skilled in the art, such as polymerase chain reaction (PCR).

25 When homologous amino acid sequences are evaluated based on functional characteristics, then a homologous amino acid sequence is considered equivalent to an amino acid sequence of the invention when the homologous sequence is essentially unable to repair (in the case of the repair defective mutant gene) or able to repair (in the case of the natural gene), DNA double-strand 30 breaks, including that which occurs at a nucleotide cleavage site

-14-

↓
CAACAG
GTTGTC
↑

5 and when the homologous amino acid sequence allows normal mitotic recombination.

This invention provides screening methods whereby genes are cloned from plasmid libraries by complementation of a recessive marker. A recipient strain such as *Saccharomyces cerevisiae* is constructed that carries a
10 recessive mutation in the gene of interest. This strain is then transformed with a plasmid, for example, pYES2 (Invitrogen, San Diego, CA) containing the wild-type genomic DNA or cDNA. The clone carrying the gene of interest can then be selected by replica plating to a medium that distinguishes mutant from wild-type phenotypes for the gene of interest. The plasmid can then be extracted from
15 the clone and the DNA studied. Several yeast vectors allow the application of complementation systems to go beyond isolation of yeast genes. Genes from a wide variety of species can be isolated using these vectors. In such systems, DNA sequences from any source are cloned into a vector and can be screened directly in yeast for activities that will complement specific yeast mutations.

20 In a preferred embodiment, the present invention uses a mutation in yeast, the *hrr25* mutation, which was identified by sensitivity to DNA double-strand breaks induced by the HO endonuclease. The genomic DNA which complements this mutation was isolated by transforming the *hrr25* strain with a DNA library and subsequently screening for methylmethane sulfonate (MMS)
25 resistance. Alternately, functional genes from a variety of mammalian species can now be cloned using the system described.

Yeast genes can be cloned by a variety of techniques, including use of purified RNA as hybridization probes, differential hybridization of regulated RNA transcripts, antibody screening, transposon mutagenesis, cross suppression
30 of mutant phenotypes, cross hybridization with heterologous CDNA or oligonucleotide probes, as well as by complementation in *E. coli*.

-15-

Minor modifications of the primary amino acid sequence may result in proteins which have substantially equivalent or enhanced activity as compared to the sequence set forth in SEQ. I.D. NO. 2. The modifications may be deliberate, as by site-directed mutagenesis, or may be spontaneous by *HRR25* producing organisms. All of these modifications are included in the invention as long as *HRR25* activity is retained. Substitution of an aspartic acid residue for a glycine acid residue at position 151 in the sequence shown in SEQ. I.D. NO. 2 identifies the mutant *hrr25*.

Antibodies provided by the present invention are immunoreactive with the mutant polypeptides and/or the naturally occurring protein kinase. Antibody which consist essentially of numerous monoclonal antibodies with different epitopic specificities, as well as distinct monoclonal antibody preparations are provided. Monoclonal antibody is made from antigen containing fragments of the polypeptide by methods well known in the art (Kohler, G. *et al.*, *Nature* 256:495, 1975; *Current Protocols in Molecular Biology*, Ausubel, F. *et al.*, ed., 1989).

The invention also discloses a method for identifying a composition which affects the activity of a polypeptide having tyrosine kinase activity. The polypeptide is capable of promoting restoration of DNA double-strand break repair activity in host cells containing the *hrr25* gene. The composition and the polypeptide are incubated in combination with host cells for a period of time and under conditions sufficient to allow the components to interact, then subsequently monitoring the change in protein kinase activity, for example, by decreased repair of DNA double-strand breaks. The DNA strand breaks are induced, for example, by a radiomimetic agent, such as methylmethane sulfonate, x-rays, or by endonuclease like *HO*. Other means of inducing double-strand breaks that are well known in the art may be employed as well.

One embodiment of the invention provides a method of treating a cell proliferative disorder associated with or *HRR25* or an *HRR25*-like protein comprising administering to a subject with the disorder, a therapeutically effective amount of reagent which modulates an *HRR25*-like protein activity. The term "cell proliferative disorder" denotes malignant as well as non-malignant cell

-16-

populations which differ from the surrounding tissue both morphologically and/or genotypically. Such disorders may be associated, for example, with abnormal expression of *HRR25*-like protein genes. "Abnormal expression" encompasses both increased or decreased levels of expression as well as expression of mutant forms such that the normal function of *HRR25*-like genes is altered. Abnormal expression also includes inappropriate temporal expression during the cell cycle or expression in an incorrect cell type. Antisense polynucleotides of the invention are useful in treating malignancies of the various organ systems. Essentially, any disorder which is etiologically linked to altered expression of *HRR25*-like genes is a candidate for treatment with a reagent of the invention. "Treatment" of cell proliferative disorder refers to increasing or decreasing populations of malignant or non-malignant cells.

As used herein, the term "modulate" envisions the suppression of *HRR25*-like protein expression or the augmentation of expression. When a cell proliferative disorder is associated with *HRR25*-like gene overexpression, appropriate reagents such as antisense or binding antibody can be introduced to a cell. This approach utilizes, for example, antisense nucleic acid and ribozymes to block translation of a specific *HRR25*-like protein mRNA, either by masking that mRNA with an antisense nucleic acid or by cleaving it with a ribozyme. Alternatively, when a cell proliferative disorder is associated with insufficient *HRR25*-like protein, a sense polynucleotide sequence (the DNA coding strand) or *HRR25*-like polypeptide can be introduced into the cell by methods known in the art.

As used herein, the term "therapeutically effective" refers to that amount of polynucleotide, antibody or polypeptide that is sufficient to ameliorate the *HRR25*-associated disorder. "Ameliorate" denotes a lessening of the detrimental effect of the *HRR25*-associated disorder in the subject receiving therapy.

Antisense nucleic acids are DNA or RNA molecules that are complementary to at least a portion of a specific mRNA molecule (Weintraub, *Scientific American*, 262:40, 1990). In the cell, the antisense nucleic acids hybridize to the corresponding mRNA, forming a double-stranded molecule. This

-17-

interferes with the translation of the mRNA since the cell will not translate a mRNA that is double-stranded. Antisense oligomers of about 15 nucleotides are preferred, since they are easily synthesized and are less likely to cause non-specific interference with translation than larger molecules when introduced into the target *HRR25* producing cell. The use of antisense methods to inhibit the *in vitro* translation of genes is well known in the art (Marcus-Sakura, *Anal. Biochem.*, 172:289, 1988).

Ribozymes are RNA molecules possessing the ability to specifically cleave other single-stranded RNA in a manner analogous to DNA restriction endonucleases. Through the modification of nucleotide sequences which encode these RNAs, it is possible to engineer molecules that recognize specific nucleotide sequences in an RNA molecule and cleave it (Cech, *J. Amer. Med. Assn.*, 260:3030, 1988). A major advantage of this approach is that, because ribosomes are sequence-specific, only mRNAs with particular sequences are inactivated.

There are two basic types of ribozymes namely, *tetrahymena*-type and "hammerhead"-type. *Tetrahymena*-type ribozymes recognize sequences which are four bases in length, while "hammerhead"-type ribozymes recognize base sequences 11-18 bases in length. The longer the recognition sequence, the greater the likelihood that sequence will occur exclusively in the target mRNA species. Consequently, hammerhead-type ribozymes are preferable to *tetrahymena*-type ribozymes for inactivating a specific mRNA species and longer recognition sequences are preferable to shorter recognition sequences.

The present invention also provides gene therapy for the treatment of cell proliferative disorders which are mediated by *HRR25*-like polypeptides. Such therapy comprises introducing into cells of subjects having the proliferative disorder, the *HRR25*-like antisense polynucleotide. Delivery of antisense polynucleotide can be achieved using a recombinant expression vector such as a chimeric virus or a colloidal dispersion system. Disorders associated with under-expression of *HRR25* can similarly be treated using gene therapy with nucleotide coding sequences.

Various viral vectors which can be utilized for gene therapy as taught herein include adenovirus, herpes virus, vaccinia, or, preferably, an RNA

-18-

virus such as a retrovirus. Preferably, the retroviral vector is a derivative of a murine or avian retrovirus. Examples of retroviral vectors in which a single foreign gene can be inserted include, but are not limited to: Moloney murine leukemia virus (MoMuLV), Harvey murine sarcoma virus (HaMuSV), murine mammary tumor virus (MuMTV), and Rous Sarcoma Virus (RSV). A number of additional retroviral vectors can incorporate multiple genes. All of these vectors can transfer or incorporate a gene for a selectable marker so that transduced cells can be identified and generated. By inserting an *HRR25*-like sequence of interest into the viral vector, along with another gene which encodes the ligand for a receptor on a specific target cell, for example, the vector is now target specific. Retroviral vectors can be made target specific by inserting, for example, a polynucleotide encoding a sugar, a glycolipid, or a protein. Preferred targeting is accomplished by using an antibody to target the retroviral vector. Those of skill in the art will know of, or can readily ascertain without undue experimentation, specific polynucleotide sequences which can be inserted into the retroviral genome to allow target specific delivery of the retroviral vector containing the *HRR25*-like antisense polynucleotide.

Since recombinant retroviruses are defective, they require assistance in order to produce infectious vector particles. This assistance can be provided, for example, by using helper cell lines that contain plasmids encoding all of the structural genes of the retrovirus under the control of regulatory sequences within the LTR. These plasmids are missing a nucleotide sequence which enables the packaging mechanism to recognize an RNA transcript for encapsidation. Helper cell lines which have deletions of the packaging signal include but are not limited to Ψ 2, PA317 and PA12, for example. These cell lines produce empty virions, since no genome is packaged. If a retroviral vector is introduced into such cells in which the packaging signal is intact, but the structural genes are replaced by other genes of interest, the vector can be packaged and vector virion produced.

Alternatively, NIH 3T3 or other tissue culture cells can be directly transfected with plasmids encoding the retroviral structural genes *gag*, *pol* and *env*, by conventional calcium phosphate transfection. These cells are then

-19-

transfected with the vector plasmid containing the genes of interest. The resulting cells release the retroviral vector into the culture medium.

Another targeted delivery system for *HRR25*-like antisense polynucleotides comprises a colloidal dispersion system. Colloidal dispersion systems include macromolecule complexes, nanocapsules, microspheres, beads, and lipid-based systems including oil-in-water emulsions, micelles, mixed micelles, and liposomes. The preferred colloidal system of this invention is a liposome. Liposomes are artificial membrane vesicles which are useful as delivery vehicles *in vitro* and *in vivo*. It has been shown that large unilamellar vesicles (LUV), which range in size from 0.2-4.0 μm can encapsulate a substantial percentage of an aqueous buffer containing large macromolecules. RNA, DNA and intact virions can be encapsulated within the aqueous interior and be delivered to cells in a biologically active form (Fraley, *et al.*, *Trends Biochem. Sci.*, 6:77, 1981). In addition to mammalian cells, liposomes have been used for delivery of polynucleotides in plant, yeast and bacterial cells. In order for a liposome to be an efficient gene transfer vehicle, the following characteristics should be present: (1) encapsulation of the genes of interest at high efficiency while not compromising their biological activity; (2) preferential and substantial binding to a target cell in comparison to non-target cells; (3) delivery of the aqueous contents of the vesicle to the target cell cytoplasm at high efficiency; and (4) accurate and effective expression of genetic information (Mannino, *et al.*, *Biotechniques*, 6:682, 1988).

The targeting of liposomes has been classified based on anatomical and mechanistic factors. Anatomical classification is based on the level of selectivity, for example, organ-specific, cell-specific, and organelle-specific. Mechanistic targeting can be distinguished based upon whether it is passive or active. Passive targeting utilizes the natural tendency of liposomes to distribute to cells of the reticulo-endothelial system (RES) in organs which contain sinusoidal capillaries. Active targeting, on the other hand, involves alteration of the liposome by coupling the liposome to a specific ligand such as a monoclonal antibody, sugar, glycolipid, or protein, or by changing the composition or size of

-20-

the liposome in order to achieve targeting to organs and cell types other than the naturally occurring sites of localization.

The surface of the targeted delivery system may be modified in a variety of ways. In the case of a liposomal targeted delivery system, lipid groups
5 can be incorporated into the lipid bilayer of the liposome in order to maintain the targeting ligand in stable association with the liposomal bilayer. Various linking groups can be used for joining the lipid chains to the targeting ligand.

In general, the compounds bound to the surface of the targeted delivery system will be ligands and receptors which will allow the targeted
10 delivery system to find and "home in" on the desired cells. A ligand may be any compound of interest which will bind to another compound, such as a receptor.

The present invention will be better understood upon consideration of the following illustrative examples wherein: Example 1 addresses isolation of *hrr25* mutant strains of *Saccharomyces cerevisiae*; Example 2 describes the
15 isolation of HRR25 DNA by complementation screening; Example 3 is drawn to characterization of the DNA and putative amino acid sequence of HRR25; Example 4 addresses microscopic analysis of HRR25 wild type and *hrr25* mutant yeast morphology; Example 5 addresses the relationship of the amino acid sequence of HRR25 and three exemplary protein kinases which are not HRR25-
20 like; Example 6 describes the isolation of DNAs encoding two *Schizosaccharomyces pombe* HRR25-like protein kinases; Example 7 is directed to isolation of DNA encoding another *Saccharomyces cerevisiae* protein, NUF1; Example 8 is drawn to isolation of DNAs encoding various eukaryotic species HRR25-like proteins including three human isoforms, CKI α 1Hu, CKI α 2Hu, and
25 CKI α 3Hu; Examples 9 and 10 are respectively directed to determination of casein kinase and both serine-threonine kinase and tyrosine kinase activities for HRR25; Example 11 is drawn to the recombinant expression of *HRR25* products and the generation of antibodies thereto; Example 12 relates to the isolation of human CKI isoforms, CKI γ 1Hu and CKI γ 2Hu; Example 13 addresses isolation of another
30 human isoform CKI δ Hu; Example 14 describes complementation of yeast CKI mutants with human CKI isoforms; and Example 15 is directed to generation of monoclonal antibodies against peptide fragments of human CKI α Hu isoforms.

-21-

The following examples are intended to illustrate but not limit the invention. While they are typical of those that might be used, other procedures known to those skilled in the art may alternatively be used.

Example 1

Isolation of *hrr25*

5 *S. cerevisiae* strain K264-5B (*MAT α ho ura3 can1^R tyr1 his7 lys2 ade5 met13 trp5 leu1 ade5*) was employed for the mutant isolation. The yeast were transformed according to standard procedures with a *URA3*-based integrating plasmid that contained a *GAL1,10*-regulated *HO* endonuclease and a transformant
10 was mutagenized to approximately 50% survival with ethyl methanesulfonate (EMS), as described (*Current Protocols in Molecular Biology, supra*). The culture was spread onto glycerol-containing rich medium (YPG, to avoid petites), colonies were allowed to form at 30°C, and plates were replicated to glucose (*HO* repressing) and galactose (*HO* inducing) media. Mutants were identified by their
15 inability to grow on galactose. Approximately 200 mutants were chosen for initial characterization and 62 maintained the *gal*- phenotype through repeated single colony purification. Among these, many were not complemented by various *gal* mutants. The remainder (25 mutants) were surveyed for overlapping DNA repair defects by determining sensitivity to ultraviolet (UV) irradiation and to methyl
20 methane sulfonate (MMS). This screening method identified five alleles of known *rad* mutations and one new mutation. This new mutation *hrr25-1* (*HO* and/or radiation repair), presented severe defects and was studied further.

A recessive DNA repair defect is conferred by *hrr25-1* that includes sensitivity to MMS. *Hrr25-1* strains also show sensitivity at 5-20 Krad X-
25 irradiation similar to that observed with mutations in the radiation repair genes *RAD50* and *RAD52* (Cole, *et al.*, *Mol.Cell.Biol.*, 9:3101, 1989). The *hrr25-1* strains are no more sensitive to UV irradiation than wild type and are not temperature sensitive for growth at 37°C. Unlike hypo- and hyper-rec *rad* mutants which have several of the *hrr25-1* phenotypes, *hrr25-1* strains undergo
30 normal mitotic recombination (Cole, *et al.*, *Mol.Cell.Biol.*, 9:3101, 1989). Spontaneous gene conversion and crossing-over were the same for homozygous

-22-

hrr25-1 and wild type strains. However, *HRR25* is required for the correct completion of meiosis. The *hrr25-1* homozygotes showed less than 1% spores (tetranucleate cells) under conditions that produced 75-80% spores in an isogenic wild type strain. The *hrr25-1* mutation could be complemented by a number of radiation sensitive mutations (*rad6*, 50, 52, 54, and 57) that present some of the *hrr25* phenotypes, suggesting that *hrr25-1* is a newly uncovered *rad*-like mutation and not one of these previously described genes. These results also indicate that *HRR25* plays a role in DNA repair and meiosis, but is not specifically required for the repair of spontaneous mitotic lesions by recombination.

10

Example 2

Isolation of *HRR25*

The *HRR25* gene was obtained by complementing for MMS sensitivity using a yeast genomic library constructed in the plasmid YCp50 (Rose, *et al.*, *Gene*, 60:237, 1987). An *hrr25-1* strain, MHML 3-36d (*ura3 hrr25*), was transformed by standard methods (Nickoloff, *et al.*, *J.Mol.Biol.*, 207:527, 1989) to uracil prototrophy, transformants were amplified on media without uracil and replicated to media containing 0.01% MMS. Among 1200 transformants, a single MMS resistant isolate was identified. Complementation for MMS sensitivity was found to segregate with the plasmid as determined by methods known in the art.

20

A 12 kb genomic fragment was identified and complementing activity was localized to a 3.1 kb *Bam*HI-*Sa*II fragment by transposon mutagenesis and subcloning. This region complemented DNA repair defects as well as meiotic deficiencies. Gene targeting experiments linked this cloned region to *hrr25-1*. Transposon insertion mutations within the *Bam*HI-*Sa*II fragment replaced into the cognate *HRR25* genomic locus did not complement *hrr25-1* for MMS sensitivity, whereas adjacent chromosomal insertions outside the complementing region segregated in repulsion when crossed against *hrr25-1*.

25

Mini-Tn10LUK transposons (Huisman, *et al.*, *Genetics*, 116:191, 1987) were used to delineate the approximate location of *HRR25* on the 12 kb *Bam*HI-*Sa*II fragment. Insertions located to the left hand 9 kb (of the 12 kb genomic fragment) did not inactivate complementation of *hrr25-1* MMS resistance

30

-23-

compared with the un-mutagenized plasmid. Two insertions, located near an *EcoRV* site in the right hand 2 kb inactivated complementation. *HRR25* complementation activity was localized to a 3.4 kb *SaII* fragment. Approximately 300 bp of this fragment (right hand side of the 12 kb) were part of the pBR322 tetracycline resistance gene (between the *BamHI* site of PBR322-based YCp50).
5 The *HRR25* open reading frame spans an internal region across an *EcoRV* site and two *BglII* sites within the right terminal 3 kb.

The DNA sequence of the 3.1 kb fragment revealed a centrally located open reading frame of 1482 nucleotide. A transposon insertion mutation
10 in this open reading frame inactivated *HRR25* complementation whereas insertions elsewhere in the 12 kb clone did not affect *HRR25* complementation. Transposon-mediated disruption of *HRR25* also revealed several phenotypes not seen with *hrr25-1*. As expected, a *Tn10*-based LUK transposon insertion (Huisman, *et al.*, *Genetics*, 116:191, 1987) into the middle of plasmid-borne *HRR25* coding region
15 inactivated complementation for MMS sensitivity. Transplacement of this insertion into the genomic *HRR25* gene revealed a severe growth defect in addition to MMS sensitivity and meiotic inviability. This severe growth defect was not observed with *hrr25-1* strains. Wild type *HRR25* strains doubled in rich media at 30°C every 80-90 minutes whereas isogenic *hrr25::LUK* strains and
20 *hrr25Δ* doubled every 9-12 hours. *hrr25-1* had a doubling time of 2-4 hours.

To determine whether the mutant phenotypes revealed by the *hrr::LUK* disruption allele represent a null phenotype, the entire *HRR25* coding sequence was deleted. Briefly, deletion of the *HRR25* coding sequence employed a *hisG::URA3::hisG* cassette (Alani, *et al.*, *Genetics*, 116:541, 1988). The 3.1
25 kb *HRR25 SaII* fragment was cloned into pBluescript (Stratagene, La Jolla, CA). This plasmid was digested with *BglII* and the two *BglII* fragments that span the entire *HRR25* gene and its flanking sequences were deleted. Into this deletion was introduced the 3.8 kb *BamHI-BglII hisG::URA3::hisG* fragment from pNKY51 to create the *hrr25Δ* allele. *SaII* digestion yielded a linearized fragment that deleted
30 the entire *HRR25* locus. Yeast carrying the deletion-disruption allele (*hrr25Δ*) showed phenotypes identical to those with the *hrr25::LUK* allele for all properties examined, including MMS sensitivity, slow growth, and the sporulation defect,

-24-

indicating that wild-type *HRR25* protein is associated with these processes and that the *hrr25::LUK* allele does not indirectly interfere with DNA repair, growth or sporulation. In direct parallel comparisons, the *hrr25::LUK* and *hrr25Δ* alleles behaved identically.

5 Yeast strain MFH14 (*MATa/MATα ura3/ura3*) was transformed with *Bgl*II-linearized YCp50-*HRR25::LUK* to uracil prototrophy, heterozygous disruption of *HRR25* was verified by Southern blot analysis, the diploid was sporulated by starvation for nitrogen and fermentable carbon sources, tetrads
10 dissected and cells allowed to germinate at 30°C for 7 days. After a normal germination period of 2 days, the severe growth defect of *hrr25::LUK* suggested that the deletion of *HRR25* was lethal. However, microscopic examination of segregants revealed that *hrr25::LUK* germinating cells grew slowly and in every case examined (20/20 tetrads), slow growth, MMS sensitivity, and uracil prototrophy co-segregated. A color variation was seen with diploid MFH14
15 segregants, due to mutations in adenine biosynthesis. MFH14 is *ade5/ADE5 ade2/ade2*. An *ade5/ade2* strain was white, while an *ADE5/ade2* strain was red.

Example 3

Sequence and Structure of the *HRR25* GENE

20 DNA sequencing of both strands of the *HRR25* gene was done by uni-directional deletions employing Sequenase (USB, Cleveland, OH) and Exo-Meth (Stratagene, La Jolla, CA) procedures as described by the manufacturers. DNA and deduced amino acid sequences are set out respectively in SEQ. I.D. NOs. 1 and 2. Figure 1A, shows the alignment of the amino acid sequences for *HRR25*, *CDC28*, *KSS1*, and *RAF1*. Figure 1B shows a schematic representation
25 of the structure of *HRR25*. The protein kinase homology is represented by a shaded region while the P/Q rich region is indicated by cross-hatchings. The mutant, *hrr25*, can be distinguished from *HRR25* by one amino acid substitution. At position 151, an aspartic acid is substituted for glycine.

30 The predicted translation product of *HRR25* revealed an unexpected feature for a *rad*-like DNA repair function. *HRR25* contains the hallmark signatures of sequence homology with the catalytic domain of serine/threonine

-25-

protein kinase superfamily members (Hanks, *et al.*, *Science*, 241:42, 1988). For comparison, the *HRR25* translation product was aligned with the catalytic domains for two subgroups of yeast protein kinases, the *CDC28/cdc2* group and the *KSS1/FUS3* group. Located between amino acids 15 and 30 is a region that contains the conserved GXGXXG region. Just C-terminal to this region is a conserved lysine and glutamic acid present in most known kinases. These regions are thought to function in the nucleotide binding and phosphotransfer steps of the kinase reaction (Hanks, *et al.*, *Science*, 241:42,1988). Between amino acid residues 120 to 150 are regions containing the HRD and DFG motifs, also found in most protein kinase family members. In addition, sequence examination of all known serine/threonine kinases indicates that *HRR25* shares some additional similarities with the *Raf/PKS/mos* subgroup (Hanks, *et al.*, *Science*, 241:42, 1988). The strongest homologies can be found in areas around the GXGXXG, DFG, and DXSXXG conserved regions in protein kinase catalytic domains.

The functional relevance of the observed sequence similarity between *HRR25* and protein kinases was studied by altering specific residues within the *HRR25* kinase domain and examining the phenotypic consequences of these changes. A lysine at position 38 (Lys³⁸) was mutated to an arginine residue by site directed mutagenesis, by methods known in the art. The mutagenic oligonucleotide SEQ. I.D. NO. 22 was:

5'-CCTGATCGATTCCAGCCTGATCGCTACTTCTTCACCACT-3'.

Lys³⁸ in *HRR25* corresponds to the lysine found in all known protein kinases, and this subdomain is involved in ATP binding. Mutations at the conserved lysine in protein kinases such as *v-src*, *v-mos*, and *DBF2* inactivate these proteins. The mutant *hrr25*-Lys³⁸ allele was incapable of complementing *hrr25-1*, *hrr25::LUK*, and *hrr25Δ* alleles for all properties examined, an indication that the *HRR25* kinase domain is required for *in vivo* function of *HRR25*.

The predicted *HRR25* translation product (SEQ. I.D. NO. 2) has a number of notable features outside the region of homology to protein kinase catalytic domains. For example, the last 100 amino acids is proline and glutamine

-26-

rich, containing 50 of these residues. Other proteins with regions rich in these two amino acids include the transcription factors *Sp1*, *jun*, and *HAP2*, steroid hormone receptors, the *S. pombe ran1* kinase, and *mak*-male germ cell-associated kinase (Courey, *et al.*, *Cell*, 55:887, 1988; Bohmann, *et al.*, *Science*, 238:1386, 1987; Roussou, *et al.*, *Mol.Cell.Biol.*, 8:2132, 1988; Arriza, *et al.*, *Science*, 237:268, 1987; Matsushime, *et al.*, *Mol.Cell.Biol.*, 10:2261, 1990). In the case of *Sp1* and *jun*, the proline-glutamine regions are involved in transactivation, whereas the P/Q region in the human mineralocorticoid receptor is thought to serve as an intramolecular bridge. This proline-glutamine region in *HRR25* might function as a structural feature for substrate interaction, or for subcellular localization. Also, the glutamine richness of this region is similar to the *opa* or M-repeat seen in the *Drosophila* and *Xenopus* *Notch/Xotch* proteins (Wharton, *et al.*, *Cell*, 40:55, 1985; Coffman, *et al.*, *Science*, 249:1438, 1990). The function of the *opa* repeat is not certain, but it is found in several *Drosophila* genes. Lastly, the sequence TKKQKY at the C-terminal end of the region homologous to protein kinases is similar to the nuclear localizing signal of SV40 large T antigen and yeast histone H2B (Silver, *et al.*, *J.Cell.Biol.*, 109:983, 1989; Moreland, *et al.*, *Mol.Cell.Biol.*, 7:4048, 1987).

Example 4

Microscopic Analysis of Germinating and Proliferating *hrr25* Cells

Photomicrographs of *HRR25* and *hrr25::LUK* colonies were taken after germination on rich medium. An MFH14 *hrr25::LUK* heterozygous transformant was dissected onto a thin film of YPD rich medium on a sterilized microscope slide and segregants were allowed to germinate under a coverslip by incubating the slide in a moist 30°C chamber. Photographs of colonies were taken after 2 days of growth. Phase contrast and DAPI staining of proliferating *HRR25Δ* and *hrr25::LUK* cells were compared. Cells were inoculated into YPD rich medium and grown at 30°C to a mid-log density of 1-3 X 10⁷ cells/ml, briefly sonicated to disrupt clumps, fixed with formaldehyde, and stained with

-27-

DAPI (Williamson, *et al.*, *Meth.Cell.Biol.*, 12:335, 1975). Many cells with *hrr25::LUK* lacked DAPI stainable nuclei.

Microscopic examination of germinating and actively growing mid-log phase *hrr25::LUK* cells revealed aberrant cellular morphologies. Transposon
5 disruption of *HRR25* resulted in large cells, and 25-40% of cells were filamentous or extended. DAPI nuclear staining (Williamson, *et al.*, *Meth.Cell.Biol.*, 12:335, 1975) of mid-log populations showed that orderly cell cycle progression in *hrr25* mutants was lost. There were a large number of cells lacking DAPI-stainable nuclei which, by single cell manipulations proved to be inviable. Consistent with
10 this nuclear segregation defect, the plating efficiency of *hrr25::LUK* haploids was also reduced to 75-80% of wild type. However, this reduction in plating efficiency is insufficient to account for the severe growth rate reduction. Plating efficiency was measured from mid-log phase cells by comparing the efficiency of colony formation on rich medium relative to the total number of cells determined
15 by hemocytometer count. Cell populations were analyzed for DNA content distribution by flow cytometric analysis following staining with propidium iodide as described (Hutter, *et al.* *J.Gen.Microbiol.*, 113:369, 1979). Cell sorting analysis showed that a large number of the cells in a haploid *hrr25::LUK* population were delayed in the cell cycle and exhibited G2 DNA content, but the
20 population was not arrested uniformly in the cell cycle.

Example 5

Sequence Comparison of *HRR25* with *CDC28*, *KSS1*, and *RAF1*

The predicted translation product of *HRR25* (SEQ. I.D. NO. 2) was
25 compared with the catalytic domains of several members of the serine/threonine protein kinase superfamily. Initial sequence comparisons employed the UWGCG programs (Devereux, *et al.*, *Nuc.Acids.Res.*, 12:387, 1984), whereas subgroup comparisons used the methods of Hanks, *et al.*, *supra*. *HRR25* contains all eleven subdomains described by Hanks, *et al.*, *supra*. Structurally similar groupings
30 were compared in the sequence comparisons. These included nonpolar chain R

-28-

groups, aromatic or ring-containing R groups, small R groups with near neutral polarity, acidic R groups, uncharged polar R groups, and basic polar R groups.

CDC28 and *KSS1* represent members of two subgroups of serine/threonine protein kinases in yeast. *CDC28* is involved in cell cycle regulation while *KSS1* acts in the regulation of the yeast mating pathway. *HRR25* shows 21% identity and 41% similarity to *CDC28* and 19% identity and 43% similarity to *KSS1* (Figure 1A). *HRR25* shows highest similarity to members of the *Raf1/PKS/Mos* family of protein kinases. Through the catalytic domain, *HRR25* shows 30% identity and 49% similarity to *Raf1*.

10

Example 6

Identification, Isolation, and Analysis of *Sc. pombe Hhp1+* and *Hhp2+* Genes

A. Isolation of the *Hhp1+* and *Hhp2+* Genes

The clones were isolated by a two-pronged approach: i) DNA-based screening methods; and ii) direct complementation in *S. cerevisiae hrr25* mutant strains. Two genes were identified (*Hhp1+* and *Hhp2+* - so named for *HRR25* Homologue from *Schizosaccharomyces pombe*). Expression of *Hhp1+* in *S. cerevisiae hrr25* mutants fully rescued all mutant defects. Expression of *Hhp2+* in *S. cerevisiae* also rescued, to varying degrees, the defects associated with *hrr25* mutations.

20

DNA-based amplification of *HRR25*-like DNAs from *Sc. pombe* genomic and CDNA sequences prepared according to Fikes, *et al.* (*Nature*, 346:291-293, 1990) was conducted using polymerase chain reaction with the following partially degenerate oligonucleotide primers:

25

- (1) Primer No. 4583 (SEQ. ID. NO. 13) representing top strand DNA encoding residues 16 through 23 of *HRR25*; [1 nmol/5 μ l], $T_m = 52^\circ\text{C}$;

-29-

- (2) Primer No. 4582 (SEQ. ID. NO. 14) representing top strand DNA encoding residues 126 through 133 of *HRR25*; [1.5 nmol/5 μ l], $T_m = 54^\circ\text{C}$;
- 5 (3) Primer No. 4589 (SEQ. ID. NO. 15) representing bottom strand DNA encoding residues 126 through 133 of *HRR25*; [0.5 nmol/5 μ l], $T_m = 54^\circ\text{C}$;
- (4) Primer No. 4590 (SEQ. ID. NO. 16) representing bottom strand DNA encoding residues 194 through 199 of *HRR25*; [2 nmol/5 μ l], $T_m = 38^\circ\text{C}$.

10 Two series of amplifications were conducted using Perkin Elmer Automated apparatus; a first series using *HRR25*-based primer Nos. 4583 and 4589 and a second series employing all four of the primers. In the first series, 30 cycles of denaturation (94°C , 1 min), annealing (48°C , 1 min), and extension (66°C , 3 min) were performed and in a final cycle, the extension time was
15 extended to 5 min. Reaction products were sized on an agarose gel revealing a prominent band of the expected size of about 306 bp. In the second series of amplifications, 30 cycles were carried out as above except that annealing and extension were carried out at 35°C and 60°C , respectively. Three major products of the expected sizes (513 bp, 180 bp, and 306 bp) were developed in both
20 genomic and CDNA libraries and were purified by preparative agarose gel electrophoresis.

Products were cloned into M13mp19 and sequenced by the dideoxy method (Maniatis, *et al.*, *Molecular Cloning: A Laboratory Manual*, 1982). Two classes of sequences were identified. A representative clone from each class was
25 radiolabelled with ^{32}P by random primed cut labeling to a specific activity of 10^6 cpm/ μg (Maniatis, *et al.*, *supra*) and used as a hybridization probe to isolate full length CDNA clones and to prove yeast genomic DNA in Southern blots and total RNA on Northern blots. Hybridization was carried out for 16 hours in a buffer

-30-

containing 6 x SSPE, 0.1% SDS, 5% dextran sulfate. Two genes were identified and designated Hhp1+ and Hhp2+ for *HRR25* Homologues from *Sc. pombe*.

For *Hhp1+*, 7 clones were identified (6 partial and 1 full length clone). For *Hhp2+*, 2 full length clones were identified. Both Southern and Northern analysis confirmed that these clones were from separate genes. These genes were sequenced using standard dideoxy method (Maniatis, *et al.*, *supra*). The nucleotide and deduced amino acid sequences for *Hhp1+* are set out in SEQ. ID. NOS. 3 and 4; the nucleotide and deduced amino acid sequences for *Hhp2+* are set out in SEQ. ID. NOS. 5 and 6.

10 B. Functional analysis of *Hhp1+* and *Hhp2+* in *S. cerevisiae hrr25* mutants.

Sc. pombe Hhp1+ and *Hhp2+* cDNAs were cloned in a location which placed them under the control of the *S. cerevisiae* alcohol dehydrogenase-1 (ADH1) promoter in a URA3-based vector pDB20 to allow for expression in *S. cerevisiae* (Fikes, *et al.*, *supra*). These resulting clones were analyzed for their ability to alter/modify the suppress phenotypes associated with the *hrr25-1* mutation and the *hrr25Δ* mutation following transformation into appropriate yeast strains by standard methods (Ito, *et al.*, *J. Bacteriol.* 153:163, 1983). Transformants were analyzed for their ability to overcome defects associated with the *hrr25* mutations (Hoekstra, *et al.*, *Science*, 253:1031, 1991). *Hhp1+* expression fully complemented *hrr25*-associated defects and was indistinguishable from wild type *HRR25* in all analyses. Complementation was analyzed for the effect on DNA repair, cell cycle progression, cellular morphology, and sporulation. *Hhp2+* complemented to a lesser degree than *Hhp1+* (its complementation level was 50%-75% that of *bona fide HRR25*). The alteration of *hrr25*-associated phenotypes was dependent upon the transformed yeast strains containing both a complementing *Sc. pombe Hhp* plasmid and having *hrr25* mutations.

The degree of amino acid homology between *HRR25* protein and *Hhp1+* protein is 73% through the kinase domain. The degree of similarity, which considers the presence of similar as well as identical amino acids, is greater than 85%. The amino acid identity of *HRR25* protein and *Hhp2+* protein is 63%

-31-

with a percent similarity score of 80%. The intraspecies comparison of *Hhp1+* protein to *Hhp2+* protein is 72% identity. This structural and complementation analysis clearly indicates that these *Sc. pombe* clones are functional homologues of the *S. cerevisiae* *HRR25*. Such a high degree of relatedness is not seen with
5 any other group of protein kinases. As a measure of comparison here, *bona fide* functional homologues (i.e., *cdc2* protein kinases from *S. cerevisiae*, *Sc. pombe*, and humans) show 40%-45% identity. Any two randomly compared protein kinases, regardless of whether the comparison is inter-or intra-species show a degree of identity of about 20%-25%.

10 C. Disruption and mutation of *Hhp1+* and *Hhp2+* in *Sc. pombe*

Mutations that inactivate or reduce the protein kinase activity of *HRR25* in *S. cerevisiae* result in a wide variety of phenotypes including: sensitivity to various forms of DNA damage, severe cell cycle delay, sensitivity to drugs
15 that affect cell cycle progression (e.g., caffeine), sensitivity to agents that affect microtubule integrity (e.g., benomyl), and sensitivity to agents that affect the integrity of replicating DNA (e.g., hydroxyurea).

Similarity, in *Sc. pombe*, inactivation of the *Hhp1+* and the *Hhp2+* genes to reduce or abolish the encoded protein kinase activity resulted in
20 cellular phenotypes that mimicked *hrr25* mutations. For example, deletion of the *Hhp1+* gene resulted in a cell cycle delay and aberrant cellular morphology, in sensitivity to DNA damaging agents like MMS, and in sensitivity to benomyl and hydroxyurea. Deletion of the *Hhp2+* gene resulted in caffeine sensitivity, benomyl sensitivity, and hydroxyurea sensitivity, amongst other defects.

25 The *Hhp1+* gene was disrupted as follows: CDNA was subcloned into the *Sc. pombe* vector pHSS19 (Hoekstra *et al.*, *Meth. Enzymol.*, 194:329, 1991), which was digested with *NheI-EcoRI*. The *Sc. pombe* URA4 gene was inserted resulting in deletion of the *Hhp1+* kinase domain. *Sc. pombe* was transformed by standard methods (Moreno, *et al.*, *Meth. Enzymol.*, 194:795,
30 1991) with the linearized DNA from the resulting plasmid construction. Stable

-32-

transformants were identified and haploid *hhp1* Δ strains were verified by standard methods (Moreno, *et al.*, Maniatis, *et al.*).

The *Hhp2+* gene was disrupted as follows: the *Hhp2+* CDNA was cloned into the *Sc. pombe* based vector, plasmid pHSS19, and was disrupted
5 by transposon shuttle mutagenesis using the mini-Tn3 transposon mTn3Leu2 (Hoekstra, *et al.*, *Meth. Enzymol supra.*). *Sc. pombe* was transformed by standard methods with the linearized DNA from the resulting plasmid construction. Stable transformants were identified and haploid *hhp2* Δ strains were verified by standard methods (see above).

10 Standard physiological methods as described for *S. cerevisiae* *HRR25* (Hoekstra, *et al.*, *Science* 253:1031, 1991) were employed to characterize *hhp* mutant strains. Phenotypic analysis revealed that both *hhp1* and *hhp2* mutants showed defects previously seen in *hrr25* mutants, including sensitivity to various DNA damaging treatments that include MMS treatment and X-ray treatment.

15 The foregoing substantiates that *Hhp1+* and *Hhp2+* are isoforms of *S. cerevisiae* *HRR25* protein kinase. These three protein kinases show high levels of sequence identity. In addition, mutations that inactivate these kinases result in very similar defects in widely divergent organisms.

20 D. Complementation of *Sc. pombe* mutant strains with the *S. cerevisiae* *HRR25* gene.

To show that *Sc. pombe* *hhp* mutants prepared as described above, were identical to *S. cerevisiae* *hrr25* mutants and to show that *HRR25*-like protein kinases with greater than 35% amino acid identity are functional homologues, the *S. cerevisiae* *HRR25* gene was introduced into a *Sc. pombe* expression vector and
25 transformed into *Sc. pombe* *hhp* mutants. The DNA sequence at the *HRR25* initiating methionine was changed into an *NdeI* site, (a silent coding alteration that maintains the reading frame but allows the *HRR25* gene to be introduced into appropriate *Sc. pombe* plasmids). This was done by a site-directed DNA change was made in the *S. cerevisiae* *HRR25* gene by standard methods using a
30 commercially available system (Bio-Rad, Cambridge, MA). The altered *HRR25* gene was ligated into the *Sc. pombe* expression plasmid, pREP 1 (Maundrell, K.

-33-

J., *Biol. Chem.* 265:10857, 1990), at an *NdeI* site and the resulting construction was transformed by standard methods into *Sc. pombe hhp* mutants. Expression of *HRR25* in *Sc. pombe* mutant strains resulted in complementation of the mutant defects as evaluated by physiological methods described by Hoekstra, *et al.* (Science, *supra*).

Example 7

Isolation and Characterization of Yeast *HRR25*-like Genes

Isolation of additional *HRR25*-like genes from *S. cerevisiae* was accomplished by performing DNA-based amplification of genomic DNA from an *S. cerevisiae* strain lacking *HRR25* coding sequences [Strain 7D of DeMaggio, *et al.* (*Proc. Natl. Acad. Sci., USA*, 89:7008-7012, 1992, incorporated herein by reference) thereby eliminating the chance of obtaining *HRR25* sequences from the amplification. The primers and amplification conditions were as in Example 6.

The resulting amplification products were cloned in M13mp19 and sequenced by dideoxy chain termination methods. Three unique classes of amplified products were identified. Two of these products respectively corresponded to the YCK1/CKI2 and YCK2/CKI1 genes of Robinson, *et al.* (*Proc. Natl. Acad. Sci. USA*, 89:28-32, 1992) and Wang, *et al.* (*Molecular Biology of the Cell*, 3:275-286, 1992). The third gene product was designated "NUF1" (for Number Four). The amplified products corresponding to NUF1 were radiolabelled as described in Example 6 and used to screen a yeast YCp50-based genomic library (ATCC, Rockville, MD). Eight clones were identified and one of these clones included approximately 4 Kb *HindIII* fragment containing the NUF1 hybridizing gene. Southern analysis revealed that NUF1 is a separate gene from *HRR25*, YCK1/CKI2, and YCK2/CKI1. The *HindIII* fragment was sequenced and revealed a protein kinase with about 65% identity to *HRR25* through its protein kinase domain. The DNA and deduced amino acid sequences for NUF1 are set out in SEQ. ID. NOS. 23 and 24.

To further characterize the NUF1 gene, the *HindIII* fragment was subcloned into the yeast plasmid YEplac112 [Gietz and Sugino, *Gene* 74:527-541

-34-

(1988)]. The resulting construct was transformed into the *hrr25Δ* deletion strain 7d and NUF1 was found to complement for *hrr25Δ* mitotic defects (e.g., NUF1 complemented for slow growth defect, aberrant morphology defect, DNA damaging agent sensitivities). Furthermore, a null mutant allele of NUF1 was
5 constructed by transposon shuttle mutagenesis and strains lacking the NUF1 gene product were found to have *hrr25Δ* mutant-like defects. In particular, like *hrr25Δ* mutants, NUF1 mutants showed slower mitotic growth rates and increased sensitivity to DNA damaging agents like MMS, UV, and X-irradiation.

Example 8

10 Identification and Isolation of Human HRR25-like Genes

Oligonucleotides derived from amino acid sequences described above in Example 6A were used to amplify cDNAs from the following sources: *Arabidopsis thaliana*, *Drosophila melanogaster*, *Xenopus*, chicken, mouse, rat,
15 and human HeLa cells. These cDNAs were obtained from reverse transcribed mRNA (Maniatis, *et al.*, *supra*) or from commercially-available cDNA libraries (Stratagene, La Jolla, CA, and Clontech, Palo Alto, CA) Amplification products of similar migration size to those obtained from *S. cerevisiae* *HRR25* and *Sc. pombe*, *Hhp1+* and *Hhp2+* genes were observed in 1.0% Agarose gels
20 (Maniatis, *et al.*, *supra*). This result indicated that *HRR25*-like genes exist in all species examined.

Isolation of full length DNAs encoding human *HRR25*-like protein kinases was accomplished by PCR amplification of human genomic DNA using unique sequence oligonucleotide primers based on portions of a bovine brain
25 casein kinase I cDNA which had been reported in Rowles, *et al.* (*Proc. Natl. Acad. Sci. USA*, 88:9548-9552, 1991) to encode a mammalian protein that was 60% homologous to *HRR25* over its catalytic domain.

A variety of primers were prepared and used in pairwise fashion including:

-35-

- (1) Primer JH21 (SEQ. ID. NO. 17) representing bovine top strand DNA bases 47-67;
- (2) Primer JH22 (SEQ. ID. NO. 18) representing bovine top strand DNA bases 223-240;
- 5 (3) Primer JH29 (SEQ. ID. NO. 19) representing bovine top strand DNA bases 604-623;
- (4) Primer JH30 (SEQ. ID. NO. 20) representing bovine top strand DNA bases 623-604; and
- 10 (5) Primer JH31 (SEQ. ID. NO. 21) representing bovine top strand DNA bases 835-817.

DNA amplification with combination of oligonucleotides JH21/JH30, JH22/JH30, and JH29/JH31 were carried out for 30 cycles with denaturation performed at 94°C for 4 min for the first cycle and for 1 min for the remaining cycle annealing at 50°C for 2 min and extension at 72°C for 4 min.

15 Products of the expected size from the three amplifications were purified on preparative acrylamide gels and labeled with ³²P using random nick translation (to a specific activity between 7 x 10⁶ cpm/μg and 1.4 x 10⁷ cpm/μg. The labelled probes were employed as a group to screen a commercial human fetal brain cDNA library (Stratagene). Hybridization was carried out for 16 hours at

20 65°C in a hybridization buffer containing 3 x SSC, 0.1% Sarkosyl, 10 x Denhart's solution and 20 mM sodium phosphate (Ph 6.8). Three washes at 65°C in 2 x SSC, 0.1% SDS were performed. Approximately 1.5 x 10⁶ plaques were screened on 30 plates using duplicate filters. Six strong positive clones were isolated, purified and converted to plasmid form according to procedures

25 recommended by the supplier of the library. Restriction digestion revealed the

-36-

following insert sizes for the six clones: clone 35A1, 1kb; clone 35B1, 1.4kb; clone 41A1, 3.7kb; clone 42A1, >4kb; clone 47A1, 3.35kb; and clone 51A1, 2.75kb. All six inserts contained sequences which could be aligned with both the DNAs and deduced protein sequence of the bovine CKI α gene. The abbreviated, partial cDNA clones 35A1 and 35B1 were not further analyzed. Clones 41A1 and 42A1 were identical except for size. Clones 42A1, 51A1, and 47A1 were redesignated as CKI α 1Hu, CKI α 2Hu, and CKI α 3Hu. The DNA and deduced amino acid sequences of the inserts are set out in SEQ. ID. NOS. 7 and 8; 9 and 10; and 11 and 12, respectively. The deduced amino acid sequence for CKI α 1Hu was identical to the reported bovine CKI α sequence. Table 1, below sets out differences in nucleotides between the bovine and human DNAs, numbered from the first base in the initiation codon, ATG.

-37-

TABLE 1
COMPARISON OF HUMAN AND BOVINE CKI α DNA

	<u>Position</u>	<u>Bovine</u>	<u>Human</u>	<u>Position</u>	<u>Bovine</u>	<u>Human</u>
	+ 9	C	T	+591	A	G
5	+ 27	A	T	+594	A	G
	+ 93	T	C	+669	A	G
	+126	G	A	+687	A	G
	+147	C	T	+690	G	A
	+186	A	G	+705	A	G
10	+255	T	C	+729	A	G
	+258	C	T	+731	C	T
	+261	G	A	+753	A	G
	+267	T	C	+771	C	G
	+279	T	G	+798	G	A
15	+285	C	T	+816	G	A
	+291	T	C	+828	C	T
	+372	C	T	+867	T	C
	+540	T	C	+870	C	T
	+555	T	C	+936	A	C
20	+558	G	A			

The CKI α 3Hu DNA also includes an insertion of 84 bases at position +454 in the coding sequence providing an intermediate extension of the CKI α 2Hu expression product by 28 amino acids. This DNA insert is not present in the bovine gene, but it encodes an amino acid sequence insert which Rowles, *et al.* designated as CKI-alpha-L. The CKI α 2Hu and CKI α 3Hu DNAs insertion at position +971 of the CKI α 1Hu DNA. This insertion is not found in any of the bovine sequences and encodes an extension of the 13 amino acids adjacent the carboxy terminal. The last two codons of the CKI α 3Hu sequences differ from

-38-

any of the bovine sequences or the sequences of CKI α 1Hu and CKI α 2Hu, causing the CKI α 3Hu expression product to terminate with a lysine, rather than a phenylalanine as found in all the other bovine and human casein kinase I sequences. The 3' flanking sequence of CKI α 3Hu DNA differs significantly from
5 that of CKI α 1Hu and CKI α 2Hu.

FIGURE 2 provides an alignment of the catalytic domain amino acid sequences of *HRR25*-like proteins whose DNAs were isolated in the above illustrative examples, including *HRR25*, *Hhp1*+, *Hhp2*+, CKI α 1Hu, CKI α 2Hu, and CKI α 3Hu as well as YCK1/CKI2, and YCK2/CKI1. Note that with the
10 exception of the CKI α 3Hu intermediate insert and the carboxy terminal region inserts of CKI α 2Hu and CKI α 3Hu, the sequences of the three human products are identical. "Common" residues are indicated in the Figure where at least 3 of the seven residues are identical at the corresponding position (the human sequences
being taken as a single sequence).

15 Like *Hhp1* + and *Hhp2* +, the three human *HRR25*-like protein kinases showed very high degrees of amino acid identity to the *HRR25* gene product (68%), establishing that these human clones were enzymatic isoforms of the yeast *HRR25* gene. The alignment of *HRR25*, *Hhp1* +, *Hhp2* +, and the human complementing-like kinase isoforms show that these enzymes share a
20 number of primary structural features that indicate that these enzymes provide comparable activities in different species. This conclusion is reached based on several lines of evidence. First, all enzymes share the common primary sequence identifiers characteristic of protein kinases. Second, the enzymes share high
degrees of amino acid identity in regions of the protein kinase domain that are not conserved in unrelated protein kinases. Finally, these enzymes share regions of
25 identity in the kinase domain which regions differ in primary sequence from other protein kinases, but are identical among the members of this isoform grouping. For example, greater than 95 % of all known protein kinases have a so-called A-P-E sequence (Alanine-Proline-Glutamate) approximately two-thirds of the way
30 through the kinase domain. *HRR25*-like protein kinases lack the A-P-E sequence and have instead a S-I/V-N sequence (Serine-Isoleucine or Valine-Asparagine). Based on this primary sequence comparison, between known protein kinases and

-39-

the protein kinases of the invention from evolutionarily divergent organisms, these enzymes of the invention are isoforms of *HRR25* protein kinase.

Example 9

Comparison of *HRR25* with a Casein Kinase

5 In all eukaryotes examined, two of the major protein kinases are casein kinase I and II (CKI and CKII, respectively). These enzymes have been found in all cell types and species examined. Both enzymes recognize Ser/Thr residues in an acidic environment in the substrate. These two protein kinases are found throughout the cell and their activities have been purified from or found to
10 be associated with cytoplasmic fractions, membranes, nuclei, mitochondria, and cytoskeleton. CKII is predominantly a nuclear enzyme, but similar studies have yet to be described for CKI.

To determine whether *HRR25* gene product might function as a casein kinase, the ability of *HRR25*-containing immunoprecipitates to
15 phosphorylate casein was studied. *HRR25*-containing immunoprecipitates from yeast were incubated with casein and phosphorylated proteins were examined.

Yeast extracts were prepared by physical disruption. Equal volumes of a cells were suspended in lysis buffer and acid-washed 0.5 mm beads were mixed, 30 second bursts were interspersed with 1 min on ice, and the extent
20 of disruption was followed microscopically. Lysis buffer contained 10 Mm sodium phosphate (Ph 7.2), 150 Mm NaCl, 1% Nonidet P-40, 1% Trasylol, 1 Mm DTT, 1 Mm benzamidine, 1 Mm phenylmethyl sulfonyl fluoride, 5 Mm EDTA, pepstatin (1 ug/ml), Pepstatin A (2 ug/ml), leupeptin (1 ug/ml), 100mM sodium vanadate, and 50 Mm NaF. Extracts were clarified by a 100,000 x g
25 centrifugation for 30 min., made to 50% (vol/vol) with glycerol, frozen in liquid nitrogen, and stored at -70 degrees C. Little loss in protein kinase activity was seen in frozen extracts over several months.

Immune complex protein kinase assays were performed on the extracts according to the methods described in Lindberg, *et al.* (*Mol. Cell. Biol.*
30 10:6316, 1991). Frozen extracts were diluted to 25% glycerol with lysis buffer

-40-

or fresh extracts were used directly. Extracts were precleared with preimmune serum and protein A-Sepharose, and then treated with immune serum (obtained as described in Example 11, *infra*, from immunization of rabbits with *E. coli*-derived type-*HRR25* fusion products). *HRR25* kinase-containing immune
5 complexes were precipitated with protein A-Sepharose. Immune complexes were washed four times with lysis buffer and twice with kinase buffer containing 15 Mm Hepes (Ph 7.4), 100 Mm NaCl, and 10 Mm MgCl₂.

Reaction mixtures of *HRR25* immunoprecipitates and heat-treated casein (300 ng/20ul reaction volume) were incubated at 30 degrees C for 5-10 min
10 and contained 10 uCi of gamma-³²P-ATP per 20 ul reaction volume. Reactions were stopped by the addition of SDS and EDTA, boiled in SDS/PAGE sample buffer and resolved in 10 % gels. Phosphoamino acid analysis was as described (Hunter *et al.*, *Proc.Natl.Acad.Sci.USA* 77:1311, 1980).

Immunoprecipitates from *HRR* + strains were able to phosphorylate
15 casein. To verify that the appropriate amino acids were phosphorylated, the phosphoamino acid composition of the *HRR25*-phosphorylated casein was examined by phosphoamino acid analysis. Samples were resolved by two-dimensional electrophoresis at Ph 1.9 and Ph 3.5. Consistent with mammalian CKI specificity, serine and threonine residues were phosphorylated. *HRR25*
20 phosphorylated serine residues on casein 3-fold greater than threonine residues. Similarly, the autophosphorylation of *HRR25* in immune complexes *in vitro* occurred on serine and threonine residues. Coupled with the high degree of sequence identity, these results suggest that *HRR25* might be a CKI isoform.

To extend and confirm that *HRR25* immunoprecipitates from yeast
25 could phosphorylate casein, several experiments were performed. *HRR25* immunoprecipitated from *E. coli* strains expressing *HRR25* (See Example 11) also showed casein kinase activity, whereas *E. coli* extracts lacking *HRR25* protein did not phosphorylate casein. *HRR25*-containing baculovirus constructs produced casein kinase activity in immunoprecipitates. Wild-type baculovirus-infected cells
30 showed <0.5% casein kinase activity under comparable conditions. The protein kinase activity from S19 cells expressing *HRR25* protein was sensitive to the same conditions that reduced or inactivated the *HRR25* protein activity from yeast

-41-

extracts. The observations that *HRR25*-dependent casein kinase activity was present in immunoprecipitates from *E. coli* cells expressing wild-type *HRR25*, in insect cells infected with *HRR25*-containing baculovirus, and in wild-type but not *hrr25* Δ mutants indicated that the *HRR25* gene product could function as a casein kinase and that the casein kinase activity in *HRR25* protein-containing immunoprecipitates was due to *HRR25* gene product.

Example 10

Analysis of Protein Kinase

Activity of *HRR25*-like Proteins

Because the predominant protein kinase activity in *E. coli* is histidine kinase, rather than serine/threonine or tyrosine kinase, those procaryotic cells provide a system for examination of *HRR25*-like protein kinase activities which is not compromised by presence of endogenous kinases. Both *HRR25* and *Hhp1* + DNAs were, therefore, expressed in the IPTG-inducible T7 gene 10-based commercial expression system (Invitrogen, San Diego, CA) using *E. coli* strain BL21 (DE3) which contains an IPTG-inducible T7 RNA polymerase and T7 lysozyme gene. See, DeMaggio, *et al.*, *Proc. Natl. Acad. Sci. USA*, 89:7008-7012, (1991). In a first series of experiments, *E. coli* lysates were prepared by inducing mid-log phase cells with IPTG for 2 hours, pelleting the cells, and preparing extracts by a freeze-thaw method using buffers described in DeMaggio, *et al.*, *supra*. Extracts were electrophoresed in polyacrylamide gels, transferred to nylon-based support membranes, and probed by Western analysis with antibodies directed against phosphotyrosine (UBI, Lake Placid, NY). These procedures revealed that *HRR25* and *Hhp1* + expressing cells contained novel tyrosine phosphorylated proteins not observed in control cells (transformed with the vector alone or with kinase inactive mutants). In a second experiment, the *HRR25* and *Hhp1* + -containing *E. coli* strains were examined for tyrosine-phosphorylated protein by a sensitive and accurate radiolabelling and phosphoamino acid procedure. To do this experiment, cells were induced with IPTG and grown in the presence of ^{32}P -orthophosphate. Radiolabelled extracts were prepared by the freeze-thaw method, electrophoresed in polyacrylamide gels,

-42-

and the gels were examined by autoradiographic methods. Novel phosphoproteins were observed in the strains expressing *HRR25* and *Hhp1*+, but not in the above controls. Phosphoproteins were examined by extracting and hydrolyzing the proteins from the gels using standard methods (Boyle, *et al.*, *Meth. Enzymol.*, 201:110, 1991). These experiments verified that *HRR25* and *Hhp1*+ could phosphorylate tyrosine, serine, and threonine residues on protein substrates.

Example 11

Recombinant Expression of HRR25

Products and Generation of Antibodies Thereto

Two different plasmid constructions were developed for expression of *HRR25* DNA in *E. coli* to generate immunogens useful in preparation of anti-*HRR25* antibodies.

The first plasmid construction involved plasmid pATH according to Koerner *et al.*, *Meth. Enzymol.*, 194:477-491 (1991). An approximately [606] base pair DNA fragment was isolated from the *HRR25* open reading frame by *Bgl* II digestion and this fragment (which encodes amino acid residues 275-476) was ligated into pATH which had been digested with *Bam*HI. The resulting plasmid encoded a fusion protein comprising the *E. coli* TrpE gene product at its amino terminus and a carboxy terminal fragment of *HRR25* at its carboxyl terminus.

Inclusion bodies were isolated from *E. coli* DH5 α (Bethesda Research Laboratories, Bethesda, MD) host cells transformed the plasmid using lysis buffers as described in Koerner *et al.*, *supra*, and were purified by polyacrylamide gel electrophoresis. The gel purified materials were then employed in the immunization of rabbits by subcutaneous injection as recommended by Harlow, *et al.*, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1988), using gel purified products with complete Freund's adjuvant for primary injections and incomplete Freund's adjuvant for subsequent injections. Serum reactivity was followed by Western blotting against the gel purified antigen. Affinity purification of serum antibodies was effected using the *E. coli*-produced antigen immobilized on a nitrocellulose membrane support.

-43-

Example 12**Isolation of CKI γ 1Hu and CKI γ 2Hu**

Additional human HRR25-like protein kinase encoding DNAs were isolated by combined DNA amplification and library screening methods.

5 Oligonucleotides based on conserved regions in HRR25-like protein kinases were used to amplify DNA segments for use as probes in screening human a cDNA library. Redundant oligonucleotides of the sequence

5'-GAR YTI MGI YTI GGI AAY YTI TA-3' (SEQ ID NO. 28)

and

10 5'-GTY TTR TTI CCI GGI CKI CCI AT-3' (SEQ ID NO. 29)

(where G, A, T, and C = standard nucleotides and R = A and G; Y = C and T; I = Inosine; M = A and C; and K = G and T) were used to amplify an approximately 540 nucleotide from a human fetal brain cDNA library (Clontech). Amplification conditions used 200 Mm Tris.Hcl (Ph 8.2), 100 Mm KCl, 60 Mm

15 (NH₄)₂SO₄, 15 Mm MgCl₂, 1% Triton X-100, 0.5 μ M of each primer, 100 ng library DNA template, 200 μ M dNTPs and 2.5 U polymerase. The reactions were performed for 30 cycles. Reactions were started with a 4 minute treatment at 94°C and all cycles were 1 minute at 94°C, 2 minutes at 5°C for annealing, and 4 minutes at 72°C for extension.

20 The amplification reaction was electrophoresed through a 1% agarose gel and the region corresponding to approximately 540 base pairs was excised and DNA was eluted using a NaI extraction and glass powder binding (GeneClean, Bio101, La Jolla, CA). The gel-purified fragment was ligated into SmaI-digested Bluescript II SK(+) and the resulting plasmid contained a partial

25 protein kinase domain that was used as a source of cDNA for library screening. Ten micrograms of this plasmid was digested with *Eco*RI and *Bam*HI to liberate the subcloned fragment and the reaction was electrophoresed through a 1% agarose gel. The approximately 540 nucleotide fragment was eluted from the gel and was radiolabelled by random primed oligonucleotide directed labelling

30 (Amersham, Arlington Heights, IL) using ³²P-dCTP as the radioactive nucleotide.

-44-

The radioactive probe was used to screen a human Manca B cell lymphoma library [Wiman, *et al.*, *Proc. Natl. Acad. Sci. (USA)* 81:6798-6802 (1984)] prepared in phage cloning vector λ gt10 prepared as follows. Poly d(A)⁺RNA was prepared from 2.8×10^8 cells of the B-cell lymphoma Manca using the "Fast Track" kit (Invitrogen). 5 μ g of RNA was used for oligo d(T) primed cDNA synthesis with the cDNA Synthesis System (Gibco BRL, Burlington, Ontario, Canada); the resulting cDNA was size selected by agarose gel electrophoresis and ligated to *Eco*RI adapters with the Ribo Clone kit (Promega, Madison, WI). Varying amounts of the adapted cDNA were ligated to *Eco*RI-digested λ gt10 with 1 unit of T4 DNA ligase (Boehringer Mannheim, Indianapolis, IN) in a commercially prepared buffer supplied by the manufacturer with the enzyme. The ligations were packaged with Gigapack packaging extracts (Stratagene) and the resulting phage pool (1.5×10^6 phage) was amplified in the C600 Hfl strain. A total of 1×10^6 phage plaques were screened by standard hybridization methods (Maniatis, *et al.*, *supra*). Hybridizations were at 65°C for 18 hours in 6X SSPE (20X SSPE is 175.3 g/l NaCl, 27.6 g/l $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$), 7.4 g/l EDTA, pH 7.4), 100 μ g/ml salmon sperm carrier DNA, 5X Denhardt Reagent (50X Denhardts is 5% ficoll, 5% polyvinyl pyrrolidone, 5% bovine serum albumin), 0.1% SDS and 5% sodium dextran sulfate. Filters were washed four times in 0.1X SSPE, 1% SDS. Each wash was at 65°C for 30 minutes. Five clones were chosen for further analysis.

DNA from these phage clones was prepared using a Qiagen lambda DNA preparation kit (Qiagen, Chatsworth, CA) and human cDNA inserts were excised by *Eco*RI digestion. These inserts were subcloned into *Eco*RI-digested plasmid Bluescript II SK(+) (Stratagene) and the inserts were sequenced using an ABI 373A automated DNA sequencer. Two of the five cDNA contained near full-length cDNAs with a polyA tail and a protein kinase open reading frame. These protein kinases were most closely related to isoforms of casein kinase I were designated CKI γ 1Hu and CKI γ 2Hu. The DNA sequences of CKI γ 1Hu and CKI γ 2Hu are set out in SEQ ID NOS: 30 and 32, respectively; the deduced amino acid sequences of CKI γ 1Hu and CKI γ 2Hu are set out in SEQ ID NOS: 31 and 33, respectively.

-45-

Example 13**Isolation of CKI δ Hu**

Human CKI δ was subcloned by first isolating the human gene from a human fetal brain library constructed in λ ZAPII (Stratagene). A 2.2 kb *Eco*RI fragment containing rat CKI δ was gel purified through 1% agarose, isolated from the gel by NaI extraction with glass powder (Bio101, La Jolla, CA), and radiolabelled by random primer methods (Boehringer Mannheim) using 32 P-dCTP. This probe was used to screen 1×10^6 plaques containing human fetal brain cDNA library. Plaque hybridization conditions were 3X SSC, 0.1% Sarkosyl, 10X Denhardts reagent, 50 μ g/ml salmon sperm DNA carrier. Hybridization was allowed to proceed for 18 hours at 65°C after which time the filters were washed 4 times for 30 minutes each at 65°C in 2X SSC, 1.0% SDS. Positive clones were identified by autoradiography at -70°C with an enhancing screen and sequenced using an automated ABI373A DNA sequencer (Applied Biosystems, Foster City, California)..

One clone was determined to encode a full length CKI δ isoform and was designated CKI δ Hu. The nucleotide sequence for CKI δ Hu is set out in SEQ ID NO: 34, and the deduced amino acid sequence is set out in SEQ ID NO: 35.

Expression of the CKI δ Hu isoform was then determined in eight different human tissues using an approximately 1.2 kb *Eco*RI fragment as a probe. CKI δ Hu mRNA levels were highest in kidney, liver and placenta cells, in contrast to the testes-specific expression of rat CKI δ demonstrated by Graves, *et al.*, [supra].

-46-

Table 2 - Sequence Homology Between CKI Isoforms

	<u>HRR25</u>	<u>Human</u> <u>CKIα1</u>	<u>Human</u> <u>CKIγ1</u>	<u>Human</u> <u>CKIγ2</u>	<u>Human</u> <u>CKIδ</u>
HRR25	100	68	50	50	65
Human CKI α 1		100	52	52	76
Human CKI γ 1			100	99	55
5 Human CKI γ 2				100	55
Human CKI δ					100

Example 14Complementation of Yeast CKI Mutantsby Human CKI Genes

10 In order to determine if CKI γ 1Hu was an isoform of yeast HRR25-like protein the gene was expressed in yeast protein kinase mutants. The cDNA was expressed under control of the yeast GAL1 promoter. The expression plasmid was a derivative of plasmid pRS305 (Stratagene) that contains the yeast GAL1 promoter. The parental plasmid with the GAL1 promoter was previously

15 described [Davis *et al.*, *Cell* 61:965-978 (1990)] and contained a *Bgl*II site adjacent to the GAL1 promoter as well as *Bam*HI and *Sac*I sites adjacent to the *Bgl*II site. This plasmid was modified by site-directed mutagenesis to contain a unique *Nco*I site between the GAL1 promoter and the *Bgl*II site. The *Nco*I site was adjacent to the GAL1 promoter such that the order of genetic elements was

20 GAL1 promoter-*Nco*I-*Bgl*II-*Bam*HI-*Sac*I. Site-directed mutagenesis (MutaGene kit, BioRad) employed the oligonucleotide

5'-CTA GAT CTA GCT AGA CCA TGG TAG TTT TTT CTC CTT GAC
G-3' (SEQ ID NO. 36)

-47-

and generated a unique *NcoI* site (underlined in SEQ ID NO: 36). The resulting plasmid was called pRS305(N) 2 μ GAL1.

To clone CKI γ 1Hu into pRS305(N) 2 μ GAL1, the CKI γ 1Hu cDNA was amplified from cDNA with oligonucleotides that would introduce an *NcoI* site at the initiating ATG and a *BamHI* site in the 3' untranslated region. The sequence of the mutagenic oligonucleotide (with the *NcoI* site underlined) for the amino terminus was

5'-CAT GCC ATG GCA CGA CCT AGT-3' (SEQ ID NO: 37).

The oligonucleotide M13rev, purchased from Stratagene (Stratagene, La Jolla, CA) was used to introduce the *BamHI* site in the 3' untranslated region. Amplification conditions used 200 mM Tris-HCl (pH 8.2), 100 mM KCl, 60 mM (NH₄)₂SO₄, 15 mM MgCl₂, 1% Triton X-100, 0.5 μ M of each primer, 100 ng template, 200 μ M of each dNTP and 2.5 units polymerase. The reactions were performed for 30 cycles. Reactions were started with a 4 minute treatment at 94°C and all cycles were 1 minute at 94°C for denaturing, 2 minutes at 50°C for annealing, and 4 minutes at 72°C for extension. The amplified product was digested with *NcoI* and *BamHI* and was cloned into *NcoI/BamHI*-digested pRS305(N) 2 μ GAL1.

Complementation of yeast CKI mutants employed yeast strains 7D (*hrr 25* Δ , *ura3-1*, *trp1-1*, *leu2-3, 112*, *his3-11,15*, *can1-100*, *ade2-1*) [DeMaggio, *et al.*, (1992) *supra*] and YI227 (*cki1D*, *cki2D*, *FOA^R*, *ade2-1*, *can1-100*, *his3-11,15*, *leu2-3,12*, *trp1-1*, *ura3-1*, *pRS415::Cki1ts*) Strain 7D lacked the HRR25 isoform of yeast CKI and strain YI227 contained a temperature sensitive allele of yeast CKI1. Yeast strains were transformed by lithium acetate-mediated transformation methods and transformants were selected on SD-leucine medium (Bio101). Controls for transformation were plasmids pRS305(N) 2 μ g GAL1 alone, plasmid pRS315 (Stratagene), and plasmid pRS315::HRR25, which contains a *SalI*-*EcoRI* genomic fragment that spans the genomic HRR25 fragment [Hoekstra *et al.*, *Science*, *supra*]. Plasmid pRS315::HRR25 was constructed by ligating a *SalI/EcoRI* genomic fragment of HRR25 into *SalI/EcoRI*-digested

-48-

pRS315. Both HRR25 and CKI γ 1Hu, when expressed in yeast mutants, are capable of fully complementing for the temperature-sensitive growth defect of CKI. In addition, CKI γ 1Hu partially suppressed a severe growth rate defect associated with HRR25 mutants. The partial suppression of HRR25 growth defects by CKI γ 1Hu was detected by a 10-20 fold greater plating efficiency relative to pRS305(N) 2 μ GAL1.

To extend the complementation analysis to additional CKI family members, the ability of other human CKI α Hu and CKI δ Hu genes to complement for the HRR25 mutant defects was examined. Human CKI α 1Hu was subcloned into plasmid pRS305(N) 2 μ GAL1 by first introducing an *Nco*I site at the initiating methionine by site-directed mutagenesis. The mutagenic oligonucleotide (with the *Nco*I site underlined) was

5'-CTA GAT CTA GCT AGA CCA TGG TAG TTT TTT CTC CTT GAC
G-3'

15 (SEQ ID NO. 38)

and mutagenesis was performed using the Mutagene kit (BioRad). The mutagenized cDNA was digested with *Nco*I and *Bgl*II and the CKI α 1Hu fragment was ligated into pRS305(n) 2 μ GAL1.

Two constructs containing the CKI δ Hu cDNA were examined for complementation. Plasmid pEC7B (containing CKI δ Hu cDNA) was used as a template for site-directed mutagenesis (MutaGene, BioRad). The mutagenic oligonucleotide

5'-GAA TCG GGC CGC CGA GAT CTC ATA TGG AGC TGA GAG TC-3'
(SEQ ID NO: 39)

25 was used to introduce *Bgl*II (underlined in SEQ ID NO: 39) and *Nde*I (in italics in SEQ ID NO: 39) sites at the initiating ATG of CKI δ Hu. One plasmid construction employed *Bgl*II/*Sac*I-digested CKI DNA from the mutagenized cDNA that was ligated into *Bgl*II/*Sac*I-digested pRS305(N) 2 μ GAL1 to produce

-49-

pRS305(CKI δ). The second plasmid construct employed *NcoI/SacI*-digested CKI δ Hu cDNA from unmutagenized pEC7B cDNA that was ligated into *NcoI/SacI*-digested pRS305(N) 2 μ GAL1 to produce pRS305(N)(CKI δ). Plasmid pRS305(CKI δ) contained the nucleotides

5 5'-CCC GGA TCT AGC AGA TCT CAT-3' (SEQ ID NO: 40)

between the GAL1 promoter and the initiating methionine of CKI δ . Plasmid pRS305(N)(CKI δ) had a near-perfect fusion between the initiating methionine of CKI δ Hu and the 3' end of GAL1. Near perfect fusion indicates that the promoter and initiating methionine codon have few or no intervening nucleic acid sequences, and therefore are approximately abutting.

10 The CKI α 1Hu and CKI δ Hu-containing plasmids were transformed into yeast strains 7D and Y1227 and were examined for their ability to complement for their mutant defects. Like CKI γ Hu, CKI α 1Hu partially complemented the growth defect associated with HRR25 mutations. CKI δ Hu was
15 able to complement for the growth defect of temperature-conditional CKI strains, for the growth defect of HRR25 mutants, and for the DNA repair defect of HRR25. The ability of CKI δ Hu to complement for mutant defects in these yeast strains was indistinguishable from yeast HRR25 or CKI genes only when the appropriate plasmid construct was employed. Plasmid pRS305(CKI δ), which
20 contained the additional 21 bases was unable to complement for any mutant phenotypes, while the near-perfect fusion in pRS305(N)(CKI δ) was fully functional. This difference was attributed to the inability of yeast to translate extended and/or CG rich leader sequences.

-50-

Example 15Generation of Monoclonal AntibodiesA. CKI α Hu Peptides

Monoclonal antibodies were raised against the following peptides.

- 5 SEQ ID NO: 41 was derived from the common amino terminus of CKI α 1Hu, CKI α 2Hu, and CKI α 3Hu, and SEQ ID NO: 42 was derived from an internal alternative splice region in CKI α 3Hu.

NH₂-ASSSGSKAEFIVGGY-COOH (SEQ ID NO: 41)

NH₂-RSMTVSTSQDPSFSGY-COOH (SEQ ID NO: 42)

- 10 These peptides were initially each coupled to bovine gamma globulin (Sigma, St Louis, MO). Five mg of gamma globulin and 5 mg of peptide were resuspended in 0.4 ml 100 mM K₂HPO₄ (pH 7.2) and to this mixture, 35 mg 1-ethyl-3(3-dimethylamino propyl)-carbodiimide-HCl (EDC, Pierce) previously dissolved in 50 μ l K₂HPO₄ (pH 7.2) was added. The reaction was allowed to proceed for 16
- 15 hr at 4°C and was quenched by addition of 0.25 ml 2 M ethanolamine and 0.25 ml acetic acid. The reaction mixture was then diluted to a final volume of 2.5 ml with PBS and desalted using Sephadex G-25M (Pharmacia) chromatography. Protein containing fractions were concentrated by centrifugal microconcentration (Amicon). Mice were then injected with 50 μ g of the coupled peptide nine times
- 20 over a period of 8 months. Antibody production was measured against the respective peptides by ELISA.

- Fusions were performed by standard methods. Briefly, a single-cell suspension was formed by grinding the spleen between the frosted ends of two glass microscope slides submerged in serum free RPMI 1640 media,
- 25 supplemented with 2 mM L-glutamine, 1 mM sodium pyruvate, 100 units/ml penicillin, and 100 μ g/ml streptomycin (RPMI) (Gibco). The cell suspension was filtered through a sterile 70-mesh Nitex cell strainer (Becton Dickinson, Parsippany, NJ), and washed twice by centrifuging at 200 g for 5 minutes and the pellet resuspended in 20 ml serum free RPMI. Thymocytes taken from 3 naive
- 30 Balb/c mice were prepared in a similar manner.

-51-

NS-1 myeloma cells, kept in log phase in RPMI with 11% fetal bovine serum (FBS) (Hyclone, Laboratories, Inc., Logan, Utah) for three days prior to fusion, were centrifuged at 200 g for 5 minutes, and the pellet was washed twice as described in the foregoing paragraph. After washing, each cell
5 suspension was brought to a final volume of 10 ml in serum free RPMI, and 10 μ l was diluted 1:100. From each dilution, 20 μ l was removed, mixed with 20 μ l 0.4% trypan blue stain in 0.85% saline (Gibco), loaded onto a hemacytometer (Baxter Healthcare Corp., Deerfield, IL) and cells counted.

Approximately 2×10^8 spleen cells were combined with 4×10^7
10 NS-1 cells, centrifuged, and the supernatant was aspirated. The cell pellet was dislodged by tapping the tube and 2 ml of 37°C PEG 1500 (50% in 75 mM Hepes, Ph 8.0) (Boehringer Mannheim) was added with stirring over the course of 1 minute, followed by adding 14 ml of serum free RPMI over 7 minutes. An additional 16 ml RPMI was added and the cells were centrifuged at 200 g for 10
15 minutes. After discarding the supernatant, the pellet was resuspended in 200 ml RPMI containing 15% FBS, 100 μ M sodium hypoxanthine, 0.4 μ M aminopterin, 16 μ M thymidine (HAT) (Gibco), 25 units/ml IL-6 (Mallinckrodt, St. Louis, MO) and 1.5×10^6 thymocytes/ml. The suspension was dispensed into ten 96-well flat bottom tissue culture plates (Corning, Essex, United Kingdom) at 200 μ l/well.
20 Cells in the plates were fed 2-3 times between fusing and screening by aspirating approximately half of the medium from each well with an 18 gauge needle (Becton Dickinson), and replenishing plating medium described above except containing 10 units/ml IL-6 and lacking thymocytes.

Fusions were screened when cell growth reached 60-80%
25 confluency (usually 7-9 days). Fusion 75 was screened by ELISA on either the common amino terminal peptide (SEQ ID NO: 41) or the internal peptide (SEQ ID NO: 42), and fusion 80 was screened on the amino terminal peptide (SEQ ID NO: 41) only. Immulon 4 plates (Dynatech, Cambridge, MA) were coated at 4°C overnight with 100 ng/well peptide in 50 mM carbonate buffer, Ph 9.6.
30 Plates were washed three times with PBS containing 0.05% Tween 20 (PBST) and 50 μ l culture supernatant was added. After incubation at 37°C for 30 minutes, and washing as above, 50 μ l horseradish peroxidase conjugated goat anti-mouse

-52-

IgG(fc) (Jackson ImmunoResearch, West Grove, PA) diluted 1:3500 in PBST was added. Plates were incubated as above, washed four times with PBST and 100 μ l substrate, consisting of 1 mg/ml *o*-phenylene diamine (Sigma) and 0.1 μ l/ml 30% H₂O₂ in 100 mM citrate, pH 4.5, was added. The color reaction was
5 stopped in 5 minutes with the addition of 50 μ l of 15% H₂SO₄. Absorbance at 490 nm was read on a plate reader (Dynatech).

Three wells from each fusion (designated 75D3G, 75C10H, 75C2g, 80G10H, 80H4F, and 80J9E) were cloned two to three times, successively, by doubling dilution in RPMI, 15% FBS, 100 μ M sodium hypoxanthine, 16 μ M
10 thymidine and 10 units/ml IL-6. Wells of clone plates were scored visually after 4 days and the number of colonies in the least dense wells were recorded. Selected wells of each cloning were tested by ELISA as above. In the final cloning, positive wells containing single colonies were expanded in RPMI with 11% FBS.

15 Three antibodies were determined to be reactive for the peptide raised against the amino terminus of CKI α Hu (80 G10H11D, 80 H12F12B, and 80 J9E10C), and three antibodies were reactive with the peptide raised against the internal fragment of CKI α 3Hu (75 D3G10A, 75 C10H1D, and 75 C2G11F). Clones 75D3G, 75C10H, 75C2G, and 80G10H were isotyped to be IgG1, clone
20 80H4F IgG3, and 80J9E IgG2a.

B. CKIHu/Thioredoxin Fusion Proteins

Expression plasmids were constructed in order to express the CKIHu isoforms as fusion proteins with thioredoxin. Specifically, the coding sequence for each isoform was amplified by PCR with primers which created a
25 5' *Xba*I restriction site and a 3' *Bam*HI site. The primer used to create the *Xba*I site for the CKI α Hu isoforms is set out in SEQ ID NO: 43 with the *Xba*I site underlined.

5'-T ACA TCT AGA ATT ATG GCG AGT AGC AGC GGC-3'
(SEQ ID NO: 43)

-53-

The primer used to create the 3' *Bam*HI site in the CKI α 1Hu coding sequence is set out in SEQ ID NO: 44, with *Bam*HI site underlined.

5'-AAT GGA TCC TTA GAA ACC TGT GGG GGT-3'
(SEQ ID NO: 44)

- 5 The primer used to create the *Bam*HI site in the CKI α 2Hu and CKI α 3Hu coding sequences is set out in SEQ ID NO: 45, with the *Bam*HI site underlined.

5'-AAT GGA TCC TTA GAA ACC TTT CAT GTT ACT CTT GGT-3'
(SEQ ID NO: 45)

- 10 The *Xba*I and *Bam*HI sites were created in the CKI δ Hu coding sequences with primers set out in SEQ ID NOS: 46 and 47, respectively.

5'-T ACA TCT AGA ATT ATG GAG CTG AGA GTC GGG-5'
(SEQ ID NO: 46)

5'-GGA TCC TCA TCG GTG CAC GAC AGA CTG-3'
(SEQ ID NO: 47)

- 15 The primers used to create the *Xba*I and *Bam*HI sites in the coding regions of the CKI γ Hu isoforms are set out in SEQ ID NO: 48 and 49.

5'-T ACA TCT AGA ATT ATG GCA CGA CCT AGT GGT CGA TCG-3'
(SEQ ID NO: 48)

- 20 5'-G GGG ATC CTA CTT CAG TAG GGG CTG-3'
(SEQ ID NO: 49)

Digestion of the resulting PCR products with *Xba*I and *Bam*HI allowed the fragments to be directionally cloned in frame at the carboxy terminus of sequences encoding thioredoxin in plasmid pTRXFUS [LeVallie, *et al.*, *Nature/Biotechnology* 11:187-193 (1993)]. The resulting expression constructions
25 contained the *laq* Iq gene, followed by the *tac*II promoter (from plasmid pMal-c2,

-54-

New England Biolabs, Beverly, MA) which drives expression of the *E.coli* thioredoxin gene fused at the amino termini of the CKI catalytic domains.

5 *E.coli* XL-1 Blue cells (Stratagene) were transformed with the individual expression plasmids by standard methods and grown at 37°C to mid-log phase. Samples were collected to serve as controls for uninduced cells and the remaining cells were induced for four hours with 0.25 mM IPTG at 37°C. Cells were then lysed and inclusion bodies in the insoluble extract from cleared lysate were used to inject mice.

C. Other CKI Peptides

10 Monoclonal antibodies were also raised against other CKI peptides coupled to bovine gamma globulin as in section A of this example. Peptides derived from the amino termini of the CKI γ Hu isoforms are set out in SEQ ID NOS: 50 and 51; peptides derived from the amino termini of bovine CKI β [Rowles, *et al.*, *supra*] are set out in SEQ ID NOS: 52 and 53; peptides derived from the amino terminus and carboxy terminus of CKI δ Hu are set out in SEQ ID NOS: 54 and 55, respectively; a peptide derived from the carboxy termini of CKI α 2Hu and CKI α 3Hu is set out in SEQ ID NO: 56; and a peptide common to all CKIHu isoforms is set out in SEQ ID NO: 57. The common CKI sequence set out in SEQ ID NO: 57 was also injected into rabbits to produce polyclonal
20 antisera.

	NH ₂ -RSGHNTRGTGSS-COOH	(SEQ ID NO: 50)
	NH ₂ -RLGHNTRGTGSS-COOH	(SEQ ID NO: 51)
	NH ₂ -SSRPKTDVLVG-COOH	(SEQ ID NO: 52)
	NH ₂ -KSDNTKSEMKHS-COOH	(SEQ ID NO: 53)
25	NH ₂ -GTDIAAGE-COOH	(SEQ ID NO: 54)
	NH ₂ -ERRDREERLR-COOH	(SEQ ID NO: 55)
	NH ₂ -TGKQTDKTKSNMKGY-COOH	(SEQ ID NO: 56)
	NH ₂ -DLLGPSLEDLFGY-COOH	(SEQ ID NO: 57)

-55-

Mice were injected with 50 μ g of the peptide/gamma globulin complex on a varying schedule over a period of eight months.

Subsequent to the filing of U.S. Patent Application Serial No. 07/728,783 on July 3, 1991, there have been numerous reports in the scientific literature of the isolation of DNAs encoding *HRR25*-like proteins. For example, Rowles, *et al.* (*Proc. Natl. Acad. Sci. USA*, 88:9548-9592, 1991) reported the purification of a bovine thymus casein kinase I (CKI) enzyme. The sequencing of tryptic fragments revealed nearly 25% of the primary sequence of the enzyme. PCR cloning resulted in development of partial clones coding for the CKI enzyme isolate and a homologue enzyme referred to as CKI- δ . Screening of bovine brain libraries with the partial clones yielded full length cDNAs for the CKI isolate (designated CKI α) and two additional homologues (CKI β and CKI γ). The deduced sequence for bovine CKI α was noted by Rowles, *et al.*, [*supra*] to be 60% homologous to *HRR25* over its catalytic domain. As noted earlier, a comparison of the bovine CKI α sequence of Rowles, *et al.* to human CKI α 1 sequence set out in SEQ. ID. NO. 7 and 8 reveals 100% homology in the catalytic domain.

As another example, Robinson, *et al.* (*Proc. Natl. Acad. Sci. USA*, 89:28-32, 1992) describes the isolation of two *Saccharomyces cerevisiae* genes, YCK1 and YCK2 which encode yeast casein kinase I homologues and also describes purification and partial sequencing of a rabbit casein kinase I from a rabbit reticulocyte lysate preparation. *HRR25* was noted to be 50% homologous to YCK1 and YCK2 and 60% homologous to the partial rabbit CKI sequence. As a further example, Wang, *et al.* (*Molecular Biology of the Cell*, 3:275-286, 1992) describes the isolation of a 54 kDa CKI from *S. cerevisiae* and the use of amino acid sequence information therefrom for cloning two yeast cDNAs encoding homologous casein kinase I proteins, CKI1 and CKI2. Comparison of the catalytic domains of the protein encoded by the CKI1 gene produced few alignments revealing greater than 20-25% homology. The closest matches were with *HRR25* (50-56%) and with the three bovine isozymes of Rowles, *et al.* (51-56%). The YCK1 sequence of Robinson, *et al.* corresponds to the CKI2

-56-

sequence of Wang, *et al.*; the YCK2 sequence corresponds to CKI1. Brockman, *et al.* (*Proc. Natl. Acad. Sci. USA*, 89:9454-9458, 1992) reported the immunopurification and sequencing of a human erythroid casein kinase I and noted that it was 62% homologous to *HRR25*. As a final example, Graves, *et al.* 5 (*J. Biol. Chem.* 265:6394-6401, 1993) reported the cloning and characterization of a casein kinase I from rat testes. This CKI, designated CKI δ , shared 76% homology at the amino acid level with CKI α isolated from bovine brain and 65% homology with *HRR25*.

While the foregoing illustrative examples are specifically directed 10 to isolation of "full length" polynucleotides encoding the *HRR25*-like proteins *HRR25*, Hhp1+, Hhp2+, CKI α 1Hu, CKI α 2Hu, CKI α 3Hu, CKI δ Hu, CKI γ 1Hu and CKI γ 2Hu, it will be readily understood that the present invention is not limited to those polynucleotides. Rather it embraces all polynucleotides which are comprehended within the class of genes encoding *HRR25*-like proteins 15 characterized protein kinase activity and by homology of 35% or more with the *HRR25* protein through the protein kinase catalytic domain. By way of example, employing information concerning the DNA sequence of *HRR25*, the procedures of Example 7 allowed the isolation partial cDNA clones of expected length from cDNA libraries derived from *Arabidopsis thaliana*, *Drosophila melanogaster*, 20 *Xenopus*, chicken, mouse, rat, and human species. These partial cDNAs may, in turn, be employed in the manner of Examples 6 and 7 to isolate full length DNA clones encoding *HRR25*-like proteins from these species. Each of these may be employed in the large scale production of the corresponding proteins by recombinant methods or for the generation of other useful polynucleotides such 25 as antisense RNAs. Recombinant expression products of such *HRR25*-like DNAs may be employed for generation of antibodies and in screens for compounds which modulate the protein kinase and/or recombination/repair functions of these enzymes. Moreover, as suggested in the publication of Rowles, *et al.*, Robinson, *et al.*, and Wang, *et al.*, multiple *HRR25*-like isozymes are expected to exist in 30 a variety of eukaryotic species as both membrane bound and cytoplasmic proteins. It appears reasonable to expect that a number of genes and gene products exist in

-57-

human species, all of which are functionally related as well as structurally related to each other and to *HRR25*.

The invention now being fully described, it will be apparent to one of ordinary skill in the art that various changes and modifications can be made
5 without departing from the spirit or scope of the invention.

-58-

SUMMARY OF SEQUENCES

SEQ ID NO: 1 is the nucleic acid sequence and the deduced amino acid of a genomic fragment encoding a yeast-derived protein kinase, *HRR25* of the present invention.

5 SEQ ID NO: 2 is the deduced amino acid sequence of a yeast-derived protein kinase *HRR25* of the present invention.

SEQ ID NO: 3 is the nucleic acid sequence (and the deduced amino acid sequence) of a genomic fragment encoding Hhp1+ of the present invention.

10 SEQ ID NO: 4 is the deduced amino acid sequence of Hhp1+ of the present invention.

SEQ ID NO: 5 is the nucleic acid sequence (and the deduced amino acid sequence) of a genomic fragment encoding Hhp2+ of the present invention.

SEQ ID NO: 6 is the deduced amino acid sequence of Hhp2+ of the present invention.

15 SEQ ID NO: 7 is the nucleic acid sequence (and the deduced amino acid sequence) of a genomic fragment encoding CK1 α 1Hu of the present invention.

SEQ ID NO: 8 is the deduced amino acid sequence of CK1 α 1Hu of the present invention.

20 SEQ ID NO: 9 is the nucleic acid sequence (and the deduced amino acid sequence) of a genomic fragment encoding CK1 α 2Hu of the present invention.

-59-

SEQ ID NO: 10 is the deduced amino acid sequence of CK1 α 2Hu of the present invention.

SEQ ID NO: 11 is the nucleic acid sequence (and the deduced amino acid sequence) of a genomic fragment encoding CK1 α 3Hu of the present invention.

SEQ ID NO: 12 is the deduced amino acid sequence of CK1 α 3Hu of the present invention.

SEQ ID NO: 13 is the primer, 4583, representing top strand DNA encoding residues 16-23 of *HRR25*.

10 SEQ ID NO: 14 is the primer, 4582, representing top strand DNA encoding residues 126-133 of *HRR25*.

SEQ ID NO: 15 is the primer, 4589, representing bottom strand DNA encoding residues 126-133 of *HRR25*.

15 SEQ ID NO: 16 is the primer, 4590, representing bottom strand DNA encoding residues 194-199 of *HRR25*.

SEQ ID NO: 17 is the primer JH21, representing bovine top strand DNA bases 47-67.

SEQ ID NO: 18 is the primer JH22, representing bovine top strand DNA bases 223-240.

20 SEQ ID NO: 19 is the primer JH29, representing bovine top strand DNA bases 604-623.

-60-

SEQ ID NO: 20 is the primer JH30, representing bovine bottom strand DNA bases 623-604.

SEQ ID NO: 21 is the primer JH31, representing bovine bottom strand DNA bases 835-817.

5 SEQ ID NO: 22 is the mutated *HRR25* kinase domain primer found on p. 33, Example 3.

SEQ ID NO: 23 is the nucleic acid sequence (and the deduced amino acid sequence) of a genomic fragment encoding NUF1 of the present invention.

10 SEQ ID NO: 24 is the deduced amino acid sequence of NUF1 of the present invention.

SEQ ID NOS: 25, 26 and 27 are the conserved motifs found on page 18.

15 SEQ ID NOS: 28 and 29 are redundant oligonucleotides, based on conserved regions of *HRR25*-like proteins, used to amplify a probe from a human cDNA library.

SEQ ID NO: 30 is the nucleotide sequence of the CKI γ 1Hu gene.

SEQ ID NO: 31 is the deduced amino acid sequence of the CKI γ 1Hu protein.

20 SEQ ID NO: 32 is the nucleotide sequence of the CKI γ 2Hu gene.

-61-

SEQ ID NO: 33 is the deduced amino acid sequence of the CKI γ 2Hu protein.

SEQ ID NO: 34 is the nucleic acid sequence for CKI δ Hu.

SEQ ID NO: 35 is the deduced amino acid sequence for CKI δ Hu.

5 SEQ ID NO: 36 is the mutagenic oligonucleotide used to generate an *Nco*I restriction site in expression plasmid pRS305.

SEQ ID NO: 37 is the mutagenic oligonucleotide used to generate an *Nco*I restriction site in CKI γ 1.

10 SEQ ID NO: 38 is the mutagenic oligonucleotide used to create an *Nco*I restriction site in human CKI α .

SEQ ID NO: 39 is the mutagenic oligonucleotide used to introduce a *Bgl*II restriction site in CKI δ .

15 SEQ ID NO: 40 is the intervening nucleic acids sequence between the GAL1 promoter and initiating methionine codon in the CKI δ expression plasmid.

SEQ ID NOS: 41 and 42 are amino terminal and internal peptide fragments of CKI α isoforms to generate monoclonal antibodies.

SEQ ID NO: 43 is the primer used to create a *Xba*I restriction site in CKI α Hu coding sequences.

20 SEQ ID NO: 44 is the primer used to create a *Bam*HI restriction site in the CKI α 1Hu coding sequence.

-62-

SEQ ID NO: 45 is the primer used to create a *Bam*HI restriction site in the CKI α 2Hu and CKI α 3Hu coding sequences.

SEQ ID NO: 46 is the primer used to create a *Xba*I restriction site in the CKI δ Hu coding sequence.

5 SEQ ID NO: 47 is the primer used to create a *Bam*HI restriction site in the CKI δ Hu coding sequence.

SEQ ID NO: 48 is the primer used to create a *Xba*I restriction site in the CKI γ 1Hu and CKI γ 2Hu coding sequences.

10 SEQ ID NO: 49 is the primer used to create a *Bam*HI restriction site in the CKI γ 1Hu and CKI γ 2Hu coding sequences.

SEQ ID NO: 50 is an amino terminal peptide fragment of CKI γ Hu coupled to bovine gamma globulin and used to generate monoclonal antibodies in mice.

15 SEQ ID NO: 51 is an amino terminal peptide fragment of CKI γ Hu coupled to bovine gamma globulin and used to generate monoclonal antibodies in mice.

SEQ ID NO: 52 is an amino terminal peptide fragment of bovine CKI β coupled to bovine gamma globulin and used to generate monoclonal antibodies in mice.

20 SEQ ID NO: 53 is an amino terminal peptide fragment of bovine CKI β coupled to bovine gamma globulin and used to generate monoclonal antibodies in mice.

-63-

SEQ ID NO: 54 is an amino terminal peptide fragment of CKI δ Hu coupled to bovine gamma globulin and used to generate monoclonal antibodies in mice.

5 SEQ ID NO: 55 is a carboxy terminal peptide fragment of CKI δ Hu coupled to bovine gamma globulin and used to generate monoclonal antibodies in mice.

SEQ ID NO: 56 is an carboxy terminal peptide fragment of CKI α 2Hu and CKI α 3Hu coupled to bovine gamma globulin and used to generate monoclonal antibodies in mice.

10 SEQ ID NO: 57 is an internal terminal peptide fragment common to all human CKI isoforms coupled to bovine gamma globulin and used to generate monoclonal antibodies in mice.

- 64 -

SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: The Salk Institute For Biological Studies
- (ii) TITLE OF INVENTION: Protein Kinases
- (iii) NUMBER OF SEQUENCES: 57
- (iv) CORRESPONDENCE ADDRESS:
 - (A) ADDRESSEE: Marshall, O'Toole, Gerstein, Murray & Borun
 - (B) STREET: 233 South Wacker Drive, 6300 Sears Tower
 - (C) CITY: Chicago
 - (D) STATE: Illinois
 - (E) COUNTRY: USA
 - (F) ZIP: 60606-6402
- (v) COMPUTER READABLE FORM:
 - (A) MEDIUM TYPE: Floppy disk
 - (B) COMPUTER: IBM PC compatible
 - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 - (D) SOFTWARE: PatentIn Release #1.0, Version #1.25
- (vi) CURRENT APPLICATION DATA:
 - (A) APPLICATION NUMBER: 08/008,001
 - (B) FILING DATE: 21-JAN-1993
 - (C) CLASSIFICATION: C12N 1/21
- (vii) PRIOR APPLICATION DATA:
 - (A) APPLICATION NUMBER: US 07/728,783
 - (B) FILING DATE: 03-JUL-1991
- (viii) ATTORNEY/AGENT INFORMATION:
 - (A) NAME: Noland, Greta E.
 - (B) REGISTRATION NUMBER: 35,302
 - (C) REFERENCE/DOCKET NUMBER: 27866/31853
- (ix) TELECOMMUNICATION INFORMATION:
 - (A) TELEPHONE: 312-474-6300
 - (B) TELEFAX: 312-474-0448
 - (C) TELEX: 25-3856

(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 3098 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (vii) IMMEDIATE SOURCE:
 - (B) CLONE: Protein Kinase

-65-

(ix) FEATURE:

(A) NAME/KEY: CDS
(B) LOCATION: 879..2360

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GTCGACTCGC CAATCACCAA GTTCTTATCC CACATCCGAC CAGTGTCTGA GTCATGGTTT	60
ACCACCACCA TACCATCGCT GGTCAATTTGT AAATCCGTTT CTATTACATC AGCACCTGCT	120
GCATAAGCCT TCTCAAATGC TAGTAGCGTA TTTTCAGGAT ATCTTGCTTT AAAAGCTCTG	180
TGGCCCACAA TTTCACCAT CCTCGTGTCC TTGTTGTTAT CTTACACTTC TTATTTATCA	240
ATAACACTAG TAACATCAAC AACACCAATT TTATATCTCC CTTAATTGTA TACTAAAAGA	300
TCTAAACCAA TTCGGTATTG TCCTCGATAC GGCATGCGTA TAAAGAGATA TAATTAAAAG	360
AGGTTATAGT CACGTGATGC AGATTACCCG CAACAGTACC ACAAATGGA TACCATCTAA	420
TTGCTATAAA AGGCTCCTAT ATACGAATAA CTACCACTGG ATCGACGATT ATTTCTGCGC	480
AATCATATAC CACTGTGAAG AGTTACTGCA ACTCTCGCTT TGTTTCAACG CTTCTTCCCG	540
TCTGTGTATT TACTACTAAT AGGCAGCCCA CGTTTGAATT TCTTTTTTTC TGGAGAATTT	600
TTGGTGCAAC GAGGAAAAGG AGACGAAGAA AAAAAGTTGA AACACGACCA CATATATGGA	660
ACGTGGTTGA AATACAAAGA GAAGAAAGGT TCGACACTCG AGGAAAGCAT TTGGTGTTGA	720
AAACACATCT TAGTAGCATC TTTAAACCTC TGTTGGGTAC TTAGAAAAAT ATTTCCAGAC	780
TTCAAGGATA AAAAAAGTCG AAAAGTTACG ACATATTCGA CCAAAAAAAA AAACCAAAAA	840
GAAAAGATAT ATTTATAGAA AGGATACATT AAAAAGAG ATG GAC TTA AGA GTA	893
Met Asp Leu Arg Val	
1 5	
GGA AGG AAA TTT CGT ATT GGC AGG AAG ATT GGG AGT GGT TCC TTT GGT	941
Gly Arg Lys Phe Arg Ile Gly Arg Lys Ile Gly Ser Gly Ser Phe Gly	
10 15 20	
GAC ATT TAC CAC GGC ACG AAC TTA ATT AGT GGT GAA GAA GTA GCC ATC	989
Asp Ile Tyr His Gly Thr Asn Leu Ile Ser Gly Glu Glu Val Ala Ile	
25 30 35	
AAG CTG GAA TCG ATC AGG TCC AGA CAT CCT CAA TTG GAC TAT GAG TCC	1037
Lys Leu Glu Ser Ile Arg Ser Arg His Pro Gln Leu Asp Tyr Glu Ser	
40 45 50	
CGC GTC TAC AGA TAC TTA AGC GGT GGT GTG GGA ATC CCG TTC ATC AGA	1085
Arg Val Tyr Arg Tyr Leu Ser Gly Gly Val Gly Ile Pro Phe Ile Arg	
55 60 65	
TGG TTT GGC AGA GAG GGT GAA TAT AAT GCT ATG GTC ATC GAT CTT CTA	1133
Trp Phe Gly Arg Glu Gly Glu Tyr Asn Ala Met Val Ile Asp Leu Leu	
70 75 80 85	
GGC CCA TCT TTG GAA GAT TTA TTC AAC TAC TGT CAC AGA AGG TTC TCC	1181
Gly Pro Ser Leu Glu Asp Leu Phe Asn Tyr Cys His Arg Arg Phe Ser	
90 95 100	
TTT AAG ACG GTT ATC ATG CTG GCT TTG CAA ATG TTT TGC CGT ATT CAG	1229
Phe Lys Thr Val Ile Met Leu Ala Leu Gln Met Phe Cys Arg Ile Gln	
105 110 115	

-66-

TAT ATA CAT GGA AGG TCG TTC ATT CAT AGA GAT ATC AAA CCA GAC AAC	1277
Tyr Ile His Gly Arg Ser Phe Ile His Arg Asp Ile Lys Pro Asp Asn	
120 125 130	
TTT TTA ATG GGG GTA GGA CGC CGT GGT AGC ACC GTT CAT GTT ATT GAT	1325
Phe Leu Met Gly Val Gly Arg Arg Gly Ser Thr Val His Val Ile Asp	
135 140 145	
TTC GGT CTA TCA AAG AAA TAC CGA GAT TTC AAC ACA CAT CGT CAT ATT	1373
Phe Gly Leu Ser Lys Lys Tyr Arg Asp Phe Asn Thr His Arg His Ile	
150 155 160 165	
CCT TAC AGG GAG AAC AAG TCC TTG ACA GGT ACA GCT CGT TAT GCA AGT	1421
Pro Tyr Arg Glu Asn Lys Ser Leu Thr Gly Thr Ala Arg Tyr Ala Ser	
170 175 180	
GTC AAT ACG CAT CTT GGA ATA GAG CAA AGT AGA AGA GAT GAC TTA GAA	1469
Val Asn Thr His Leu Gly Ile Glu Gln Ser Arg Arg Asp Asp Leu Glu	
185 190 195	
TCA CTA GGT TAT GTC TTG ATC TAT TTT TGT AAG GGT TCT TTG CCA TGG	1517
Ser Leu Gly Tyr Val Leu Ile Tyr Phe Cys Lys Gly Ser Leu Pro Trp	
200 205 210	
CAG GGT TTG AAA GCA ACC ACC AAG AAA CAA AAG TAT GAT CGT ATC ATG	1565
Gln Gly Leu Lys Ala Thr Thr Lys Lys Gln Lys Tyr Asp Arg Ile Met	
215 220 225	
GAA AAG AAA TTA AAC GTT AGC GTG GAA ACT CTA TGT TCA GGT TTA CCA	1613
Glu Lys Lys Leu Asn Val Ser Val Glu Thr Leu Cys Ser Gly Leu Pro	
230 235 240 245	
TTA GAG TTT CAA GAA TAT ATG GCT TAC TGT AAG AAT TTG AAA TTC GAT	1661
Leu Glu Phe Gln Glu Tyr Met Ala Tyr Cys Lys Asn Leu Lys Phe Asp	
250 255 260	
GAG AAG CCA GAT TAT TTG TTC TTG GCA AGG CTG TTT AAA GAT CTG AGT	1709
Glu Lys Pro Asp Tyr Leu Phe Leu Ala Arg Leu Phe Lys Asp Leu Ser	
265 270 275	
ATT AAA CTA GAG TAT CAC AAC GAC CAC TTG TTC GAT TGG ACA ATG TTG	1757
Ile Lys Leu Glu Tyr His Asn Asp His Leu Phe Asp Trp Thr Met Leu	
280 285 290	
CGT TAC ACA AAG GCG ATG GTG GAG AAG CAA AGG GAC CTC CTC ATC GAA	1805
Arg Tyr Thr Lys Ala Met Val Glu Lys Gln Arg Asp Leu Leu Ile Glu	
295 300 305	
AAA GGT GAT TTG AAC GCA AAT AGC AAT GCA GCA AGT GCA AGT AAC AGC	1853
Lys Gly Asp Leu Asn Ala Asn Ser Asn Ala Ala Ser Ala Ser Asn Ser	
310 315 320 325	
ACA GAC AAC AAG TCT GAA ACT TTC AAC AAG ATT AAA CTG TTA GCC ATG	1901
Thr Asp Asn Lys Ser Glu Thr Phe Asn Lys Ile Lys Leu Leu Ala Met	
330 335 340	
AAG AAA TTC CCC ACC CAT TTC CAC TAT TAC AAG AAT GAA GAC AAA CAT	1949
Lys Lys Phe Pro Thr His Phe His Tyr Tyr Lys Asn Glu Asp Lys His	
345 350 355	
AAT CCT TCA CCA GAA GAG ATC AAA CAA CAA ACT ATC TTG AAT AAT AAT	1997
Asn Pro Ser Pro Glu Glu Ile Lys Gln Gln Thr Ile Leu Asn Asn Asn	
360 365 370	
GCA GCC TCT TCT TTA CCA GAG GAA TTA TTG AAC GCA CTA GAT AAA GGT	2045
Ala Ala Ser Ser Leu Pro Glu Glu Leu Leu Asn Ala Leu Asp Lys Gly	
375 380 385	

-67-

ATG GAA AAC TTG AGA CAA CAG CAG CCG CAG CAG CAG GTC CAA AGT TCG Met Glu Asn Leu Arg Gln Gln Gln Pro Gln Gln Gln Val Gln Ser Ser 390 395 400 405	2093
CAG CCA CAA CCA CAG CCC CAA CAG CTA CAG CAG CAA CCA AAT GGC CAA Gln Pro Gln Pro Gln Pro Gln Gln Leu Gln Gln Gln Pro Asn Gly Gln 410 415 420	2141
AGA CCA AAT TAT TAT CCT GAA CCG TTA CTA CAG CAG CAA CAA AGA GAT Arg Pro Asn Tyr Tyr Pro Glu Pro Leu Leu Gln Gln Gln Gln Arg Asp 425 430 435	2189
TCT CAG GAG CAA CAG CAG CAA GTT CCG ATG GCT ACA ACC AGG GCT ACT Ser Gln Glu Gln Gln Gln Gln Val Pro Met Ala Thr Thr Arg Ala Thr 440 445 450	2237
CAG TAT CCC CCA CAA ATA AAC AGC AAT AAT TTT AAT ACT AAT CAA GCA Gln Tyr Pro Pro Gln Ile Asn Ser Asn Asn Phe Asn Thr Asn Gln Ala 455 460 465	2285
TCT GTA CCT CCA CAA ATG AGA TCT AAT CCA CAA CAG CCG CCT CAA GAT Ser Val Pro Pro Gln Met Arg Ser Asn Pro Gln Gln Pro Pro Gln Asp 470 475 480 485	2333
AAA CCA GCT GGC CAG TCA ATT TGG TTG TAAGCAACAT ATATTGCTCA Lys Pro Ala Gly Gln Ser Ile Trp Leu 490	2380
AAACGCACAA AAATAAACAT ATGTATATAT AGACATACAC ACACACATAT ATATATATAT	2440
ATTATTATTA TTATTTACAT ATACGTACAC ACAATTCCAT ATCGAGTTAA TATATACAAT	2500
TCTGGCCTTC TTACCTAAAA AGATGATAGC TAAAAGAACC ACTTTTTTTTA TGCATTTTTT	2560
TCTTCGGGAA GGAAATTAAG GGGGAGCGGA GCACCTCTTG GCCAATTTGT TTTTTTTTTA	2620
TGTAATAAAG GGCTAACGAT CGAAGATCAA TCACGAATAT TGGACGGTTT TAAAGGAGGG	2680
CCTCTGAGAA GACAGCATCA ATTCGTATTT TCGATAATTA ACTTGCCTTA TAGTGTCTGA	2740
TTAGGAAACA ATCACGAGAC GATAACGACG GAATACCAAG GAAGTTTGTG CAAATATACA	2800
GCCGGCACAA ACAGCAGCTT CACTCAGGTT AACTCACATA CTGTTGAAAA TTGTCGGTAT	2860
GGAATTCGTT GCAGAAAGGG CTCAGCCAGT TGGTCAAACA ATCCAGCAGC AAAATGTTAA	2920
TACTTACGGG CAAGGCGTCC TACAACCGCA TCATGATTTA CAGCAGCGAC AACACAACA	2980
ACAGCAGCGT CAGCATCAAC AACTGCTGAC GTCTCAGTTG CCCAGAAAT CTCTCGTATC	3040
CAAAGGCAAA TATACACTAC ATGACTTCCA GATTATGAGA ACGCTTGGTA CTGGATCC	3098

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 494 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Asp Leu Arg Val Gly Arg Lys Phe Arg Ile Gly Arg Lys Ile Gly
1 5 10 15

-68-

Ser Gly Ser Phe Gly Asp Ile Tyr His Gly Thr Asn Leu Ile Ser Gly
 20 25 30
 Glu Glu Val Ala Ile Lys Leu Glu Ser Ile Arg Ser Arg His Pro Gln
 35 40 45
 Leu Asp Tyr Glu Ser Arg Val Tyr Arg Tyr Leu Ser Gly Gly Val Gly
 50 55 60
 Ile Pro Phe Ile Arg Trp Phe Gly Arg Glu Gly Glu Tyr Asn Ala Met
 65 70 75 80
 Val Ile Asp Leu Leu Gly Pro Ser Leu Glu Asp Leu Phe Asn Tyr Cys
 85 90 95
 His Arg Arg Phe Ser Phe Lys Thr Val Ile Met Leu Ala Leu Gln Met
 100 105 110
 Phe Cys Arg Ile Gln Tyr Ile His Gly Arg Ser Phe Ile His Arg Asp
 115 120 125
 Ile Lys Pro Asp Asn Phe Leu Met Gly Val Gly Arg Arg Gly Ser Thr
 130 135 140
 Val His Val Ile Asp Phe Gly Leu Ser Lys Lys Tyr Arg Asp Phe Asn
 145 150 155 160
 Thr His Arg His Ile Pro Tyr Arg Glu Asn Lys Ser Leu Thr Gly Thr
 165 170 175
 Ala Arg Tyr Ala Ser Val Asn Thr His Leu Gly Ile Glu Gln Ser Arg
 180 185 190
 Arg Asp Asp Leu Glu Ser Leu Gly Tyr Val Leu Ile Tyr Phe Cys Lys
 195 200 205
 Gly Ser Leu Pro Trp Gln Gly Leu Lys Ala Thr Thr Lys Lys Gln Lys
 210 215 220
 Tyr Asp Arg Ile Met Glu Lys Lys Leu Asn Val Ser Val Glu Thr Leu
 225 230 235 240
 Cys Ser Gly Leu Pro Leu Glu Phe Gln Glu Tyr Met Ala Tyr Cys Lys
 245 250 255
 Asn Leu Lys Phe Asp Glu Lys Pro Asp Tyr Leu Phe Leu Ala Arg Leu
 260 265 270
 Phe Lys Asp Leu Ser Ile Lys Leu Glu Tyr His Asn Asp His Leu Phe
 275 280 285
 Asp Trp Thr Met Leu Arg Tyr Thr Lys Ala Met Val Glu Lys Gln Arg
 290 295 300
 Asp Leu Leu Ile Glu Lys Gly Asp Leu Asn Ala Asn Ser Asn Ala Ala
 305 310 315 320
 Ser Ala Ser Asn Ser Thr Asp Asn Lys Ser Glu Thr Phe Asn Lys Ile
 325 330 335
 Lys Leu Leu Ala Met Lys Lys Phe Pro Thr His Phe His Tyr Tyr Lys
 340 345 350
 Asn Glu Asp Lys His Asn Pro Ser Pro Glu Glu Ile Lys Gln Gln Thr
 355 360 365

-69-

Ile Leu Asn Asn Asn Ala Ala Ser Ser Leu Pro Glu Glu Leu Leu Asn
 370 375 380

Ala Leu Asp Lys Gly Met Glu Asn Leu Arg Gln Gln Gln Pro Gln Gln
 385 390 395 400

Gln Val Gln Ser Ser Gln Pro Gln Pro Gln Pro Gln Gln Leu Gln Gln
 405 410 415

Gln Pro Asn Gly Gln Arg Pro Asn Tyr Tyr Pro Glu Pro Leu Leu Gln
 420 425 430

Gln Gln Gln Arg Asp Ser Gln Glu Gln Gln Gln Gln Val Pro Met Ala
 435 440 445

Thr Thr Arg Ala Thr Gln Tyr Pro Pro Gln Ile Asn Ser Asn Asn Phe
 450 455 460

Asn Thr Asn Gln Ala Ser Val Pro Pro Gln Met Arg Ser Asn Pro Gln
 465 470 475 480

Gln Pro Pro Gln Asp Lys Pro Ala Gly Gln Ser Ile Trp Leu
 485 490

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2469 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vii) IMMEDIATE SOURCE:

- (B) CLONE: Protein Kinase

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 113..1207

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

AATATTTCAA GCTATACCAA GCATACAATC AACTCCAAGC TTCGAGCGGC CGCCAGTGTG 60

CTCTAAAGGA AAAAGCGAGT GCCTTTAGCC TTAAAAGCGT TATAATATTA TT ATG 115
 Met
 1

GCT TTG GAC CTC CGG ATT GGG AAC AAG TAT CGC ATT GGT CGT AAA ATT 163
 Ala Leu Asp Leu Arg Ile Gly Asn Lys Tyr Arg Ile Gly Arg Lys Ile
 5 10 15

GGC AGT GGA TCT TTC GGA GAC ATT TAT CTT GGG ACT AAT GTC GTT TCT 211
 Gly Ser Gly Ser Phe Gly Asp Ile Tyr Leu Gly Thr Asn Val Val Ser
 20 25 30

GGT GAA GAG GTC GCT ATC AAG CTA GAA TCA ACT CGT GCT AAA CAC CCT 259
 Gly Glu Glu Val Ala Ile Lys Leu Glu Ser Thr Arg Ala Lys His Pro
 35 40 45

CAA TTG GAG TAT GAA TAC AGA GTT TAT CGC ATT TTG TCA GGA GGG GTC 307
 Gln Leu Glu Tyr Glu Tyr Arg Val Tyr Arg Ile Leu Ser Gly Gly Val
 50 55 60 65

-70-

GGA ATC CCG TTT GTT CGT TGG TTC GGT GTA GAA TGT GAT TAC AAC GCT	355
Gly Ile Pro Phe Val Arg Trp Phe Gly Val Glu Cys Asp Tyr Asn Ala	
70 75 80	
ATG GTG ATG GAT TTA TTG GGT CCT TCG TTG GAA GAC TTG TTT AAT TTT	403
Met Val Met Asp Leu Leu Gly Pro Ser Leu Glu Asp Leu Phe Asn Phe	
85 90 95	
TGC AAT CGA AAG TTT TCT TTG AAA ACA GTT CTT CTC CTT GCG GAC CAG	451
Cys Asn Arg Lys Phe Ser Leu Lys Thr Val Leu Leu Leu Ala Asp Gln	
100 105 110	
CTC ATT TCT CGA ATT GAA TTC ATT CAT TCA AAA TCT TTT CTT CAT CGT	499
Leu Ile Ser Arg Ile Glu Phe Ile His Ser Lys Ser Phe Leu His Arg	
115 120 125	
GAT ATT AAG CCT GAT AAC TTT TTA ATG GGA ATA GGT AAA AGA GGA AAT	547
Asp Ile Lys Pro Asp Asn Phe Leu Met Gly Ile Gly Lys Arg Gly Asn	
130 135 140 145	
CAA GTT AAC ATA ATT GAT TTC GGA TTG GCT AAG AAG TAT CGT GAT CAC	595
Gln Val Asn Ile Ile Asp Phe Gly Leu Ala Lys Lys Tyr Arg Asp His	
150 155 160	
AAA ACT CAC CTG CAC ATT CCT TAT CGC GAG AAC AAG AAT CTT ACA GGT	643
Lys Thr His Leu His Ile Pro Tyr Arg Glu Asn Lys Asn Leu Thr Gly	
165 170 175	
ACT GCA CGC TAT GCT AGC ATC AAT ACT CAT TTA GGT ATT GAA CAA TCC	691
Thr Ala Arg Tyr Ala Ser Ile Asn Thr His Leu Gly Ile Glu Gln Ser	
180 185 190	
CGC CGT GAT GAC CTC GAA TCT TTA GGT TAT GTG CTC GTC TAC TTT TGT	739
Arg Arg Asp Asp Leu Glu Ser Leu Gly Tyr Val Leu Val Tyr Phe Cys	
195 200 205	
CGT GGT AGC CTG CCT TGG CAG GGA TTG AAG GCT ACC ACG AAA AAG CAA	787
Arg Gly Ser Leu Pro Trp Gln Gly Leu Lys Ala Thr Thr Lys Lys Gln	
210 215 220 225	
AAG TAT GAA AAG ATT ATG GAG AAG AAG ATC TCT ACG CCT ACA GAG GTC	835
Lys Tyr Glu Lys Ile Met Glu Lys Lys Ile Ser Thr Pro Thr Glu Val	
230 235 240	
TTA TGT CGG GGA TTC CCT CAG GAG TTC TCA ATT TAT CTC AAT TAC ACG	883
Leu Cys Arg Gly Phe Pro Gln Glu Phe Ser Ile Tyr Leu Asn Tyr Thr	
245 250 255	
AGA TCT TTA CGT TTC GAT GAC AAA CCT GAT TAC GCC TAC CTT CGC AAG	931
Arg Ser Leu Arg Phe Asp Asp Lys Pro Asp Tyr Ala Tyr Leu Arg Lys	
260 265 270	
CTT TTC CGA GAT CTT TTT TGT CGG CAA TCT TAT GAG TTT GAC TAT ATG	979
Leu Phe Arg Asp Leu Phe Cys Arg Gln Ser Tyr Glu Phe Asp Tyr Met	
275 280 285	
TTT GAT TGG ACC TTG AAG AGA AAG ACT CAA CAA GAC CAA CAA CAT CAG	1027
Phe Asp Trp Thr Leu Lys Arg Lys Thr Gln Gln Asp Gln Gln His Gln	
290 295 300 305	
CAG CAA TTA CAG CAA CAA CTG TCT GCA ACT CCT CAA GCT ATT AAT CCG	1075
Gln Gln Leu Gln Gln Gln Leu Ser Ala Thr Pro Gln Ala Ile Asn Pro	
310 315 320	
CCG CCA GAG AGG TCT TCA TTT AGA AAT TAT CAA AAA CAA AAC TTT GAT	1123
Pro Pro Glu Arg Ser Ser Phe Arg Asn Tyr Gln Lys Gln Asn Phe Asp	
325 330 335	

-71-

GAA AAA GGC GGA GAC ATT AAT ACA ACC GTT CCT GTT ATA AAT GAT CCA 1171
 Glu Lys Gly Gly Asp Ile Asn Thr Thr Val Pro Val Ile Asn Asp Pro
 340 345 350

TCT GCA ACC GGA GCT CAA TAT ATC AAC AGA CCT AAT TGATTAGCCT 1217
 Ser Ala Thr Gly Ala Gln Tyr Ile Asn Arg Pro Asn
 355 360 365

TTCATATTAT TATTATATAG CATGGGCACA TTATTTTTAT ATTTTCTTCT CATCTGGAGT 1277

CTTCCAATAC TTGCCTTTTA TCCTCCAGAC GTCCTTTAAT TTTGTTGATA GCGCAGGGCT 1337

TTTTCCTTGG GATGGCGAAA GTTACTTTGC TTATAGTTTA TTGAGGGTTC ATAGCTTATT 1397

TGGCTGAAGA TCTTGTGTTG ACTTAAATTC TATGCTAACC TCATGATCAT ATCCTCATTA 1457

TGGCAAGTTT TGGTGAAAAA TTTTSTAATA TTAGTACATT TGCTAATAAT ACATTGTTGTA 1517

TTTGTSTTTTA CTACCTGTGA ATCTATTCAT ACATTATCAT ATATGTTTCG AGCCAGGAAC 1577

AGAAAAAAGT GAGAGAATTT TCTGCAGAAA TGATCATAAT TTTATCTTCG CTTAACACGA 1637

ATCCTGGTGA CAGATTATCG TGGTTTAAAG CCTTTTTTTT ACGACGCCAT AAGCAAATTG 1697

GTTACTTTTT TATGTGTGAT GAGCCTTGGG GTTAAATCTA ATTAGAAGGC ATTGCATTCA 1757

TATACTTTTA ATAATATATT ATCAGCTATT TGCTGCTTTT CTTTATAGAT ACCGTCTTTT 1817

CCAAGCTGAA CTCATTTAAT CAGCGTCGTT TAACCTTAGG ATGCTTAAGA TCGGTTTAAA 1877

TTCAATGACT TAATGCTCGA GGGATGAATG GTTGTSTTTA GTTCGTGTTT TGGGTGCATG 1937

ATCTCGTGCT TGACTGTTTT ATTGAAGCGT TCATTTTCATG AAGTGTCTTT CGATGTTGTT 1997

CACACTTCTG TTTGCTAAAT ATAATAAATA TTTTGCTTTT CACTTTAGAG CACACTGGCG 2057

GCCGCTCGAA GCTTTGGACT TCTTCGCCAT TGGTCAAGTC TCCAATCAAG GTTGTGCGCT 2117

TGTCTACCTT GCCAGAAATT TACGAAAAGA TGGAAAAGGG ATCCAAATCG TTGGTAGATA 2177

CTTGTTGACA CTTCTAAATA AGCGAATTTT TTATGATTTA TGATTTTTAT TATTAAATAA 2237

GTTATAAAAA AAATAAGGTA TACAAATTTT AAAGTGACTC TTAGGTTTTA AAACGAAAAT 2297

TCTTATTCTT GAGTAACTCT TTCCTGTAGG TCAGGTTGCT TTCTCAGGTA TAGCATGAGG 2357

TCGCTCTTAT TGACCACACC TCTACCGGCA TGCCGAGCAA ATGCCTGCAA ATCGCTCCCC 2417

ATTTACCCCA ATTGTAGATA TGCTAACTCC AGCAATGAGC CGATGAATCT CC 2469

(2) INFORMATION FOR SEQ ID NO:4:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 365 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met Ala Leu Asp Leu Arg Ile Gly Asn Lys Tyr Arg Ile Gly Arg Lys
 1 5 10 15

Ile Gly Ser Gly Ser Phe Gly Asp Ile Tyr Leu Gly Thr Asn Val Val
 20 25 30

Ser	Gly	Glu	Glu	Val	Ala	Ile	Lys	Leu	Glu	Ser	Thr	Arg	Ala	Lys	His
		35					40						45		
Pro	Gln	Leu	Glu	Tyr	Glu	Tyr	Arg	Val	Tyr	Arg	Ile	Leu	Ser	Gly	Gly
	50					55					60				
Val	Gly	Ile	Pro	Phe	Val	Arg	Trp	Phe	Gly	Val	Glu	Cys	Asp	Tyr	Asn
65					70					75					80
Ala	Met	Val	Met	Asp	Leu	Leu	Gly	Pro	Ser	Leu	Glu	Asp	Leu	Phe	Asn
				85					90					95	
Phe	Cys	Asn	Arg	Lys	Phe	Ser	Leu	Lys	Thr	Val	Leu	Leu	Leu	Ala	Asp
		100						105					110		
Gln	Leu	Ile	Ser	Arg	Ile	Glu	Phe	Ile	His	Ser	Lys	Ser	Phe	Leu	His
		115					120					125			
Arg	Asp	Ile	Lys	Pro	Asp	Asn	Phe	Leu	Met	Gly	Ile	Gly	Lys	Arg	Gly
	130					135					140				
Asn	Gln	Val	Asn	Ile	Ile	Asp	Phe	Gly	Leu	Ala	Lys	Lys	Tyr	Arg	Asp
145					150					155					160
His	Lys	Thr	His	Leu	His	Ile	Pro	Tyr	Arg	Glu	Asn	Lys	Asn	Leu	Thr
				165					170					175	
Gly	Thr	Ala	Arg	Tyr	Ala	Ser	Ile	Asn	Thr	His	Leu	Gly	Ile	Glu	Gln
			180					185					190		
Ser	Arg	Arg	Asp	Asp	Leu	Glu	Ser	Leu	Gly	Tyr	Val	Leu	Val	Tyr	Phe
		195					200					205			
Cys	Arg	Gly	Ser	Leu	Pro	Trp	Gln	Gly	Leu	Lys	Ala	Thr	Thr	Lys	Lys
	210					215					220				
Gln	Lys	Tyr	Glu	Lys	Ile	Met	Glu	Lys	Lys	Ile	Ser	Thr	Pro	Thr	Glu
225					230					235					240
Val	Leu	Cys	Arg	Gly	Phe	Pro	Gln	Glu	Phe	Ser	Ile	Tyr	Leu	Asn	Tyr
				245					250					255	
Thr	Arg	Ser	Leu	Arg	Phe	Asp	Asp	Lys	Pro	Asp	Tyr	Ala	Tyr	Leu	Arg
			260					265					270		
Lys	Leu	Phe	Arg	Asp	Leu	Phe	Cys	Arg	Gln	Ser	Tyr	Glu	Phe	Asp	Tyr
		275					280					285			
Met	Phe	Asp	Trp	Thr	Leu	Lys	Arg	Lys	Thr	Gln	Gln	Asp	Gln	Gln	His
	290					295					300				
Gln	Gln	Gln	Leu	Gln	Gln	Gln	Leu	Ser	Ala	Thr	Pro	Gln	Ala	Ile	Asn
305					310					315					320
Pro	Pro	Pro	Glu	Arg	Ser	Ser	Phe	Arg	Asn	Tyr	Gln	Lys	Gln	Asn	Phe
				325					330					335	
Asp	Glu	Lys	Gly	Gly	Asp	Ile	Asn	Thr	Thr	Val	Pro	Val	Ile	Asn	Asp
			340					345					350		
Pro	Ser	Ala	Thr	Gly	Ala	Gln	Tyr	Ile	Asn	Arg	Pro	Asn			
		355					360					365			

-73-

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1989 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vii) IMMEDIATE SOURCE:

- (B) CLONE: Protein Kinase

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 50..1249

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```

CCGCCAGTGT GCTCTAAAGG TCATCTCTGT GAATTAGAAT CTTAGCAAA ATG ACG      55
                                         Met Thr
                                         1

GTT GTT GAC ATT AAG ATT GGT AAT AAA TAT CGT ATA GGT AGA AAA ATT      103
Val Val Asp Ile Lys Ile Gly Asn Lys Tyr Arg Ile Gly Arg Lys Ile
      5                      10                      15

GGT TCT GGC TCC TTT GGT CAA ATT TAC CTG GGA TTA AAT ACG GTA AAT      151
Gly Ser Gly Ser Phe Gly Gln Ile Tyr Leu Gly Leu Asn Thr Val Asn
      20                      25                      30

GGA GAA CAA GTT GCT GTG AAA TTG GAG CCT TTA AAG GCT CGT CAT CAT      199
Gly Glu Gln Val Ala Val Lys Leu Glu Pro Leu Lys Ala Arg His His
      35                      40                      45                      50

CAG TTA GAA TAT GAG TTT CGT GTG TAT AAT ATT CTT AAA GGA AAT ATT      247
Gln Leu Glu Tyr Glu Phe Arg Val Tyr Asn Ile Leu Lys Gly Asn Ile
      55                      60                      65

GGC ATA CCC ACA ATT CGC TGG TTC GGT GTA ACC AAT AGT TAT AAT GCT      295
Gly Ile Pro Thr Ile Arg Trp Phe Gly Val Thr Asn Ser Tyr Asn Ala
      70                      75                      80

ATG GTC ATG GAT TTA TTA GGC CCT TCT CTG GAA GAT TTA TTC TGC TAT      343
Met Val Met Asp Leu Leu Gly Pro Ser Leu Glu Asp Leu Phe Cys Tyr
      85                      90                      95

TGT GGA AGA AAG TTT ACT CTT AAA ACG GTT CTT TTA CTT GCT GAT CAA      391
Cys Gly Arg Lys Phe Thr Leu Lys Thr Val Leu Leu Leu Ala Asp Gln
      100                      105                      110

CTC ATC AGT CGC ATT GAA TAT GTT CAC TCC AAG TCA TTC TTA CAT CGA      439
Leu Ile Ser Arg Ile Glu Tyr Val His Ser Lys Ser Phe Leu His Arg
      115                      120                      125                      130

GAC ATT AAG CCT GAT AAT TTT TTA ATG AAG AAG CAC AGC AAT GTT GTT      487
Asp Ile Lys Pro Asp Asn Phe Leu Met Lys Lys His Ser Asn Val Val
      135                      140                      145

ACG ATG ATT GAC TTC GGA TTG GCG AAA AAA TAC AGG GAT TTT AAA ACT      535
Thr Met Ile Asp Phe Gly Leu Ala Lys Lys Tyr Arg Asp Phe Lys Thr
      150                      155                      160

```

-74-

CAT GTT CAT ATT CCA TAT CGA GAT AAT AAG AAT CTT ACG GGA ACG GCT His Val His Ile Pro Tyr Arg Asp Asn Lys Asn Leu Thr Gly Thr Ala 165 170 175	583
CGA TAT GCT AGT ATT AAC ACC CAT ATT GGT ATT GAA CAA TCT CGC CGT Arg Tyr Ala Ser Ile Asn Thr His Ile Gly Ile Glu Gln Ser Arg Arg 180 185 190	631
GAT GAC CTC GAA TCG TTA GGT TAT GTT TTA CTT TAT TTT TGT CGC GGC Asp Asp Leu Glu Ser Leu Gly Tyr Val Leu Leu Tyr Phe Cys Arg Gly 195 200 205 210	679
AGT TTG CCC TGG CAA GGC TTA CAA GCT GAT ACA AAG GAG CAA AAG TAT Ser Leu Pro Trp Gln Gly Leu Gln Ala Asp Thr Lys Glu Gln Lys Tyr 215 220 225	727
CAA CGG ATA CGT GAT ACC AAG ATT GGC ACT CCT TTG GAA GTC CTT TGC Gln Arg Ile Arg Asp Thr Lys Ile Gly Thr Pro Leu Glu Val Leu Cys 230 235 240	775
AAA GGT CTT CCC GAA GAG TTT ATC ACT TAC ATG TGT TAC ACT CGT CAG Lys Gly Leu Pro Glu Glu Phe Ile Thr Tyr Met Cys Tyr Thr Arg Gln 245 250 255	823
CTT TCG TTT ACC GAG AAG CCA AAC TAT GCT TAT TTG AGA AAG CTG TTT Leu Ser Phe Thr Glu Lys Pro Asn Tyr Ala Tyr Leu Arg Lys Leu Phe 260 265 270	871
CGT GAT TTA CTT ATT CGT AAA GGA TAC CAG TAT GAC TAT GTT TTT GAC Arg Asp Leu Leu Ile Arg Lys Gly Tyr Gln Tyr Asp Tyr Val Phe Asp 275 280 285 290	919
TGG ATG ATA TTA AAA TAC CAA AAG CGA GCT GCT GCT GCT GCC GCC GCT Trp Met Ile Leu Lys Tyr Gln Lys Arg Ala Ala Ala Ala Ala Ala 295 300 305	967
TCT GCT ACA GCA CCT CCA CAG GTT ACA TCT CCT ATG GTG TCA CAA ACT Ser Ala Thr Ala Pro Pro Gln Val Thr Ser Pro Met Val Ser Gln Thr 310 315 320	1015
CAA CCG GTT AAT CCC ATT ACT CCT AAT TAT TCA TCC ATT CCC TTA CCT Gln Pro Val Asn Pro Ile Thr Pro Asn Tyr Ser Ser Ile Pro Leu Pro 325 330 335	1063
GCT GAG CGG AAT CCA AAG ACT CCA CAA TCT TTC TCC ACT AAT ATT GTT Ala Glu Arg Asn Pro Lys Thr Pro Gln Ser Phe Ser Thr Asn Ile Val 340 345 350	1111
CAA TGT GCT TCT CCC TCA CCT CTT CCT CTC TCC TTT CGT TCT CCT GTT Gln Cys Ala Ser Pro Ser Pro Leu Pro Leu Ser Phe Arg Ser Pro Val 355 360 365 370	1159
CCC AAC AAA GAT TAT GAA TAC ATT CCA TCT TCG TTG CAA CCT CAA TAC Pro Asn Lys Asp Tyr Glu Tyr Ile Pro Ser Ser Leu Gln Pro Gln Tyr 375 380 385	1207
AGT GCT CAA CTG AGG CGT GTT TTA GAT GAA GAA CCA GCT CCT Ser Ala Gln Leu Arg Arg Val Leu Asp Glu Glu Pro Ala Pro 390 395 400	1249
TGATTTTTTG ACTTTACTTT TCATCAATTC CTCTCTTACA CTACGTCTTT TAGTCTTAAA	1309
TTCCAAACCA TCTGTTGACG TTTTAAAGTT CCACAAATAT CTTTAATAAT TCCTGGCTTT	1369
CTTTTTTGTC TATGGATGGC CGGATTGCTA CACTAATACA CTTTGAGGTT TAGCTATTGT	1429
TTTGAGCTAT TCCATTTTGC CTAGAAGTTG AGTTTAAATG CCTTCTTTT AAATAGACAT	1489

-75-

ATTGTGTAAA CCTCATACAT GCTTTACTGA AAAGACATAA TTAGAGGACA AAATTTAAAT	1549
CGTGCTGTTT GTTTATATTC AGCTCGTTCC GGTCAAGTTC TTGCCAAAGA ATTGAGTCAG	1609
TCGTGCTATT CATTTCTAAA TTTCTTCTTC CCAGAATTTT ATTTTATTGT TTTCGTTCCC	1669
CATTGGTTCT TACATTCCGT TTTTATTCAA AACTGAAAAG TTTGTACCTC CATTGCTAGA	1729
AGTAATATAC ACAAGGAGCA TGTTCCTTTT TTTACACTAT CATTGCGTG GCTCTAAACC	1789
AGTCTTTATT GCCTACCTTT GCAATAAAAG ATATAATATC AATTGCATAA GAAATAATTC	1849
ATTAATAAAT GATAAATTTT ATCGATTAAA TAAAAAATAA AAACCTTTAGA GCTTTAGAGC	1909
ACAACTGGCG GCCGCTCGAA GCTTTGGACT TCTTCGCCAT TGGTCAAGTC TCAATCAAGG	1969
TTGTCGGGCTT GTCTACCTTC	1989

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 400 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Met	Thr	Val	Val	Asp	Ile	Lys	Ile	Gly	Asn	Lys	Tyr	Arg	Ile	Gly	Arg	1	5	10	15
Lys	Ile	Gly	Ser	Gly	Ser	Phe	Gly	Gln	Ile	Tyr	Leu	Gly	Leu	Asn	Thr	20	25	30	
Val	Asn	Gly	Glu	Gln	Val	Ala	Val	Lys	Leu	Glu	Pro	Leu	Lys	Ala	Arg	35	40	45	
His	His	Gln	Leu	Glu	Tyr	Glu	Phe	Arg	Val	Tyr	Asn	Ile	Leu	Lys	Gly	50	55	60	
Asn	Ile	Gly	Ile	Pro	Thr	Ile	Arg	Trp	Phe	Gly	Val	Thr	Asn	Ser	Tyr	65	70	75	80
Asn	Ala	Met	Val	Met	Asp	Leu	Leu	Gly	Pro	Ser	Leu	Glu	Asp	Leu	Phe	85	90	95	
Cys	Tyr	Cys	Gly	Arg	Lys	Phe	Thr	Leu	Lys	Thr	Val	Leu	Leu	Leu	Ala	100	105	110	
Asp	Gln	Leu	Ile	Ser	Arg	Ile	Glu	Tyr	Val	His	Ser	Lys	Ser	Phe	Leu	115	120	125	
His	Arg	Asp	Ile	Lys	Pro	Asp	Asn	Phe	Leu	Met	Lys	Lys	His	Ser	Asn	130	135	140	
Val	Val	Thr	Met	Ile	Asp	Phe	Gly	Leu	Ala	Lys	Lys	Tyr	Arg	Asp	Phe	145	150	155	160
Lys	Thr	His	Val	His	Ile	Pro	Tyr	Arg	Asp	Asn	Lys	Asn	Leu	Thr	Gly	165	170	175	
Thr	Ala	Arg	Tyr	Ala	Ser	Ile	Asn	Thr	His	Ile	Gly	Ile	Glu	Gln	Ser	180	185	190	

-76-

Arg	Arg	Asp	Asp	Leu	Glu	Ser	Leu	Gly	Tyr	Val	Leu	Leu	Tyr	Phe	Cys
		195					200					205			
Arg	Gly	Ser	Leu	Pro	Trp	Gln	Gly	Leu	Gln	Ala	Asp	Thr	Lys	Glu	Gln
	210					215					220				
Lys	Tyr	Gln	Arg	Ile	Arg	Asp	Thr	Lys	Ile	Gly	Thr	Pro	Leu	Glu	Val
225				230						235					240
Leu	Cys	Lys	Gly	Leu	Pro	Glu	Glu	Phe	Ile	Thr	Tyr	Met	Cys	Tyr	Thr
			245						250					255	
Arg	Gln	Leu	Ser	Phe	Thr	Glu	Lys	Pro	Asn	Tyr	Ala	Tyr	Leu	Arg	Lys
			260					265					270		
Leu	Phe	Arg	Asp	Leu	Leu	Ile	Arg	Lys	Gly	Tyr	Gln	Tyr	Asp	Tyr	Val
		275					280					285			
Phe	Asp	Trp	Met	Ile	Leu	Lys	Tyr	Gln	Lys	Arg	Ala	Ala	Ala	Ala	Ala
	290					295					300				
Ala	Ala	Ser	Ala	Thr	Ala	Pro	Pro	Gln	Val	Thr	Ser	Pro	Met	Val	Ser
305					310					315					320
Gln	Thr	Gln	Pro	Val	Asn	Pro	Ile	Thr	Pro	Asn	Tyr	Ser	Ser	Ile	Pro
				325					330					335	
Leu	Pro	Ala	Glu	Arg	Asn	Pro	Lys	Thr	Pro	Gln	Ser	Phe	Ser	Thr	Asn
			340					345					350		
Ile	Val	Gln	Cys	Ala	Ser	Pro	Ser	Pro	Leu	Pro	Leu	Ser	Phe	Arg	Ser
		355					360					365			
Pro	Val	Pro	Asn	Lys	Asp	Tyr	Glu	Tyr	Ile	Pro	Ser	Ser	Leu	Gln	Pro
	370				375						380				
Gln	Tyr	Ser	Ala	Gln	Leu	Arg	Arg	Val	Leu	Asp	Glu	Glu	Pro	Ala	Pro
385					390					395					400

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1210 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vii) IMMEDIATE SOURCE:

- (B) CLONE: Protein Kinase

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 173..1147

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

GGCGGTGATC AGTTCCTC TGCTGATTCT GGGCCGAAC CCGGTAAAGG CCTCCGTGTT	60
CCGTTTCCTG CCGCCCTCCT CCGTAGCCTT GCCTAGTGTA GGAGCCCCGA GGCCTCCGTC	120

-77-

CTCTTCCCAG AGGTGTCGGG GCTTGCCCCA GCCTCCATCT TCGTCTCTCA GG ATG	175
Met	
1	
GCG AGT AGC AGC GGC TCC AAG GCT GAA TTC ATT GTC GGA GGG AAA TAT	223
Ala Ser Ser Ser Gly Ser Lys Ala Glu Phe Ile Val Gly Gly Lys Tyr	
5 10 15	
AAA CTG GTA CGG AAG ATC GGG TCT GGC TCC TTC GGG GAC ATC TAT TTG	271
Lys Leu Val Arg Lys Ile Gly Ser Gly Ser Phe Gly Asp Ile Tyr Leu	
20 25 30	
GCG ATC AAC ATC ACC AAC GGC GAG GAA GTG GCA GTG AAG CTA GAA TCT	319
Ala Ile Asn Ile Thr Asn Gly Glu Glu Val Ala Val Lys Leu Glu Ser	
35 40 45	
CAG AAG GCC AGG CAT CCC CAG TTG CTG TAC GAG AGC AAG CTC TAT AAG	367
Gln Lys Ala Arg His Pro Gln Leu Leu Tyr Glu Ser Lys Leu Tyr Lys	
50 55 60 65	
ATT CTT CAA GGT GGG GTT GGC ATC CCC CAC ATA CGG TGG TAT GGT CAG	415
Ile Leu Gln Gly Gly Val Gly Ile Pro His Ile Arg Trp Tyr Gly Gln	
70 75 80	
GAA AAA GAC TAC AAT GTA CTA GTC ATG GAT CTT CTG GGA CCT AGC CTC	463
Glu Lys Asp Tyr Asn Val Leu Val Met Asp Leu Leu Gly Pro Ser Leu	
85 90 95	
GAA GAC CTC TTC AAT TTC TGT TCA AGA AGG TTC ACA ATG AAA ACT GTA	511
Glu Asp Leu Phe Asn Phe Cys Ser Arg Arg Phe Thr Met Lys Thr Val	
100 105 110	
CTT ATG TTA GCT GAC CAG ATG ATC AGT AGA ATT GAA TAT GTG CAT ACA	559
Leu Met Leu Ala Asp Gln Met Ile Ser Arg Ile Glu Tyr Val His Thr	
115 120 125	
AAG AAT TTT ATA CAC AGA GAC ATT AAA CCA GAT AAC TTC CTA ATG GGT	607
Lys Asn Phe Ile His Arg Asp Ile Lys Pro Asp Asn Phe Leu Met Gly	
130 135 140 145	
ATT GGG CGT CAC TGT AAT AAG TTA TTC CTT ATT GAT TTT GGT TTG GCC	655
Ile Gly Arg His Cys Asn Lys Leu Phe Leu Ile Asp Phe Gly Leu Ala	
150 155 160	
AAA AAG TAC AGA GAC AAC AGG ACA AGG CAA CAC ATA CCA TAC AGA GAA	703
Lys Lys Tyr Arg Asp Asn Arg Thr Arg Gln His Ile Pro Tyr Arg Glu	
165 170 175	
GAT AAA AAC CTC ACT GGC ACT GCC CGA TAT GCT AGC ATC AAT GCA CAT	751
Asp Lys Asn Leu Thr Gly Thr Ala Arg Tyr Ala Ser Ile Asn Ala His	
180 185 190	
CTT GGT ATT GAG CAG AGT CGC CGA GAT GAC ATG GAA TCA TTA GGA TAT	799
Leu Gly Ile Glu Gln Ser Arg Arg Asp Asp Met Glu Ser Leu Gly Tyr	
195 200 205	
GTT TTG ATG TAT TTT AAT AGA ACC AGC CTG CCA TGG CAA GGG CTA AAG	847
Val Leu Met Tyr Phe Asn Arg Thr Ser Leu Pro Trp Gln Gly Leu Lys	
210 215 220 225	
GCT GCA ACA AAG AAA CAA AAA TAT GAA AAG ATT AGT GAA AAG AAG ATG	895
Ala Ala Thr Lys Lys Gln Lys Tyr Glu Lys Ile Ser Glu Lys Lys Met	
230 235 240	
TCC ACG CCT GTT GAA GTT TTA TGT AAG GGG TTT CCT GCA GAA TTT GCG	943
Ser Thr Pro Val Glu Val Leu Cys Lys Gly Phe Pro Ala Glu Phe Ala	
245 250 255	

-78-

ATG TAC TTA AAC TAT TGT CGT GGG CTA CGC TTT GAG GAA GCC CCA GAT 991
 Met Tyr Leu Asn Tyr Cys Arg Gly Leu Arg Phe Glu Glu Ala Pro Asp
 260 265 270
 TAC ATG TAT CTG AGG CAG CTA TTC CGC ATT CTT TTC AGG ACC CTG AAC 1039
 Tyr Met Tyr Leu Arg Gln Leu Phe Arg Ile Leu Phe Arg Thr Leu Asn
 275 280 285
 CAT CAA TAT GAC TAC ACA TTT GAT TGG ACA ATG TTA AAG CAG AAA GCA 1087
 His Gln Tyr Asp Tyr Thr Phe Asp Trp Thr Met Leu Lys Gln Lys Ala
 290 295 300 305
 GCA CAG CAG GCA GCC TCT TCC AGT GGG CAG GGT CAG CAG GCC CAA ACC 1135
 Ala Gln Gln Ala Ala Ser Ser Ser Gly Gln Gly Gln Gln Ala Gln Thr
 310 315 320
 CCC ACA GGT TTC TAAGCATGAA TTGAGGAACA GAAGAAGCAG AGCAGATGAT 1187
 Pro Thr Gly Phe
 325
 CGAGCAGCAT TTGTTTCTCC CAA 1210

(2) INFORMATION FOR SEQ ID NO:8:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 325 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Met Ala Ser Ser Ser Gly Ser Lys Ala Glu Phe Ile Val Gly Gly Lys
 1 5 10 15
 Tyr Lys Leu Val Arg Lys Ile Gly Ser Gly Ser Phe Gly Asp Ile Tyr
 20 25 30
 Leu Ala Ile Asn Ile Thr Asn Gly Glu Glu Val Ala Val Lys Leu Glu
 35 40 45
 Ser Gln Lys Ala Arg His Pro Gln Leu Leu Tyr Glu Ser Lys Leu Tyr
 50 55 60
 Lys Ile Leu Gln Gly Gly Val Gly Ile Pro His Ile Arg Trp Tyr Gly
 65 70 75 80
 Gln Glu Lys Asp Tyr Asn Val Leu Val Met Asp Leu Leu Gly Pro Ser
 85 90 95
 Leu Glu Asp Leu Phe Asn Phe Cys Ser Arg Arg Phe Thr Met Lys Thr
 100 105 110
 Val Leu Met Leu Ala Asp Gln Met Ile Ser Arg Ile Glu Tyr Val His
 115 120 125
 Thr Lys Asn Phe Ile His Arg Asp Ile Lys Pro Asp Asn Phe Leu Met
 130 135 140
 Gly Ile Gly Arg His Cys Asn Lys Leu Phe Leu Ile Asp Phe Gly Leu
 145 150 155 160
 Ala Lys Lys Tyr Arg Asp Asn Arg Thr Arg Gln His Ile Pro Tyr Arg
 165 170 175

-79-

Glu Asp Lys Asn Leu Thr Gly Thr Ala Arg Tyr Ala Ser Ile Asn Ala
 180 185 190
 His Leu Gly Ile Glu Gln Ser Arg Arg Asp Asp Met Glu Ser Leu Gly
 195 200 205
 Tyr Val Leu Met Tyr Phe Asn Arg Thr Ser Leu Pro Trp Gln Gly Leu
 210 215 220
 Lys Ala Ala Thr Lys Lys Gln Lys Tyr Glu Lys Ile Ser Glu Lys Lys
 225 230 235 240
 Met Ser Thr Pro Val Glu Val Leu Cys Lys Gly Phe Pro Ala Glu Phe
 245 250 255
 Ala Met Tyr Leu Asn Tyr Cys Arg Gly Leu Arg Phe Glu Glu Ala Pro
 260 265 270
 Asp Tyr Met Tyr Leu Arg Gln Leu Phe Arg Ile Leu Phe Arg Thr Leu
 275 280 285
 Asn His Gln Tyr Asp Tyr Thr Phe Asp Trp Thr Met Leu Lys Gln Lys
 290 295 300
 Ala Ala Gln Gln Ala Ala Ser Ser Ser Gly Gln Gly Gln Gln Ala Gln
 305 310 315 320
 Thr Pro Thr Gly Phe
 325

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2385 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 297..1388

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

GAATTCGAT AGTATTATGT GGAGTTCCAT TTTTATGTAT TTTTGTATG AAATATTCTA 60
 GTATAAGTAA ATATTTTATC AGAAGTATTT ACATATCTTT TTTTTTTTGA GTTTGAGAGC 120
 GCGCGTGATC AGGTTCCCT CTGCTGATTC TGGGCCCCGA ACCCCGGTAA AGGCCTCCGT 180
 GTTCCGTTTC CTGCCGCCCT CCTCCGTAGC CTTGCCTAGT GTAGGAGCCC CGAGGCCTCC 240
 GTCCTCTTCC CAGAGGTGTC GGGGCTTGGC CCCAGCCTCC ATCTTCGTCT CTCAGG 296
 ATG GCG AGT AGC AGC GGC TCC AAG GCT GAA TTC ATT GTC GGA GGG AAA 344
 Met Ala Ser Ser Ser Gly Ser Lys Ala Glu Phe Ile Val Gly Gly Lys
 1 5 10 15
 TAT AAA CTG GTA CGG AAG ATC GGG TCT GGC TCC TTC GGG GAC ATC TAT 392
 Tyr Lys Leu Val Arg Lys Ile Gly Ser Gly Ser Phe Gly Asp Ile Tyr
 20 25 30

-80-

TTG GCG ATC AAC ATC ACC AAC GGC GAG GAA GTG GCA GTG AAG CTA GAA Leu Ala Ile Asn Ile Thr Asn Gly Glu Glu Val Ala Val Lys Leu Glu 35 40 45	440
TCT CAG AAG GCC AGG CAT CCC CAG TTG CTG TAC GAG AGC AAG CTC TAT Ser Gln Lys Ala Arg His Pro Gln Leu Leu Tyr Glu Ser Lys Leu Tyr 50 55 60	488
AAG ATT CTT CAA GGT GGG GTT GGC ATC CCC CAC ATA CGG TGG TAT GGT Lys Ile Leu Gln Gly Gly Val Gly Ile Pro His Ile Arg Trp Tyr Gly 65 70 75 80	536
CAG GAA AAA GAC TAC AAT GTA CTA GTC ATG GAT CTT CTG GGA CCT AGC Gln Glu Lys Asp Tyr Asn Val Leu Val Met Asp Leu Leu Gly Pro Ser 85 90 95	584
CTC GAA GAC CTC TTC AAT TTC TGT TCA AGA AGG TTC ACA ATG AAA ACT Leu Glu Asp Leu Phe Asn Phe Cys Ser Arg Arg Phe Thr Met Lys Thr 100 105 110	632
GTA CTT ATG TTA GCT GAC CAG ATG ATC AGT AGA ATT GAA TAT GTG CAT Val Leu Met Leu Ala Asp Gln Met Ile Ser Arg Ile Glu Tyr Val His 115 120 125	680
ACA AAG AAT TTT ATA CAC AGA GAC ATT AAA CCA GAT AAC TTC CTA ATG Thr Lys Asn Phe Ile His Arg Asp Ile Lys Pro Asp Asn Phe Leu Met 130 135 140	728
GGT ATT GGG CGT CAC TGT AAT AAG TGT TTA GAA TCT CCA GTG GGG AAG Gly Ile Gly Arg His Cys Asn Lys Cys Leu Glu Ser Pro Val Gly Lys 145 150 155 160	776
AGG AAA AGA AGC ATG ACT GTT AGT ACT TCT CAG GAC CCA TCT TTC TCA Arg Lys Arg Ser Met Thr Val Ser Thr Ser Gln Asp Pro Ser Phe Ser 165 170 175	824
GGA TTA AAC CAG TTA TTC CTT ATT GAT TTT GGT TTG GCC AAA AAG TAC Gly Leu Asn Gln Leu Phe Leu Ile Asp Phe Gly Leu Ala Lys Lys Tyr 180 185 190	872
AGA GAC AAC AGG ACA AGG CAA CAC ATA CCA TAC AGA GAA GAT AAA AAC Arg Asp Asn Arg Thr Arg Gln His Ile Pro Tyr Arg Glu Asp Lys Asn 195 200 205	920
CTC ACT GGC ACT GCC CGA TAT GCT AGC ATC AAT GCA CAT CTT GGT ATT Leu Thr Gly Thr Ala Arg Tyr Ala Ser Ile Asn Ala His Leu Gly Ile 210 215 220	968
GAG CAG AGT CGC CGA GAT GAC ATG GAA TCA TTA GGA TAT GTT TTG ATG Glu Gln Ser Arg Arg Asp Asp Met Glu Ser Leu Gly Tyr Val Leu Met 225 230 235 240	1016
TAT TTT AAT AGA ACC AGC CTG CCA TGG CAA GGG CTA AAG GCT GCA ACA Tyr Phe Asn Arg Thr Ser Leu Pro Trp Gln Gly Leu Lys Ala Ala Thr 245 250 255	1064
AAG AAA CAA AAA TAT GAA AAG ATT AGT GAA AAG AAG ATG TCC ACG CCT Lys Lys Gln Lys Tyr Glu Lys Ile Ser Glu Lys Lys Met Ser Thr Pro 260 265 270	1112
GTT GAA GTT TTA TGT AAG GGG TTT CCT GCA GAA TTT GCG ATG TAC TTA Val Glu Val Leu Cys Lys Gly Phe Pro Ala Glu Phe Ala Met Tyr Leu 275 280 285	1160
AAC TAT TGT CGT GGG CTA CGC TTT GAG GAA GCC CCA GAT TAC ATG TAT Asn Tyr Cys Arg Gly Leu Arg Phe Glu Glu Ala Pro Asp Tyr Met Tyr 290 295 300	1208

-81-

CTG AGG CAG CTA TTC CGC ATT CTT TTC AGG ACC CTG AAC CAT CAA TAT Leu Arg Gln Leu Phe Arg Ile Leu Phe Arg Thr Leu Asn His Gln Tyr 305 310 315 320	1256
GAC TAC ACA TTT GAT TGG ACA ATG TTA AAG CAG AAA GCA GCA CAG CAG Asp Tyr Thr Phe Asp Trp Thr Met Leu Lys Gln Lys Ala Ala Gln Gln 325 330 335	1304
GCA GCC TCT TCC AGT GGG CAG GGT CAG CAG GCC CAA ACC CCC ACA GGC Ala Ala Ser Ser Ser Gly Gln Gly Gln Gln Ala Gln Thr Pro Thr Gly 340 345 350	1352
AAG CAA ACT GAC AAA ACC AAG AGT AAC ATG AAA GGT TAGTAGCCAA Lys Gln Thr Asp Lys Thr Lys Ser Asn Met Lys Gly 355 360	1398
GAACCAAGTG ACGTTACAGG GAAAAAATTG AATACAAAT TGGGTAATTC ATTTCTAACA	1458
GTGTTAGATC AAGGAGGTGG TTTTAAAATA CATAAAAATT TGGCTCTGCG TTAAAAAATA	1518
AAAAGACGTC CTTGGAAAAT TTGACTACTA ACTTTAAACC CAAATGTCCT TGTTTCATATA	1578
TATGTATATG TATTTGTATA TACATATATG TGTGTATATT TATATCATT CTCTTGGGAT	1638
TTTGGGTCAT TTTTAAACA ACTGCATCTT TTTTACTCAT TCATTAACCC CCTTTCCAAA	1698
AATTTGGTGT TGGGAATATA ATATAATCAA TCAATCCAAA ATCCTAGACC TAACACTTGT	1758
TGATTTCTAA TAATGAATTT GGTTAGCCAT ATTTTGACTT TATTCAGAC TAACAATGTT	1818
AAGATTTTTT ATTTGCGATG TTAATGCTTT AGCATTTAAA ATGGAAAATT GTGAACATGT	1878
TGTAATTTCA AGAGGTGAGT TTGGCATTAC CCCCAGAGTG TCTATCTTCT CAGTTGCAGA	1938
GCATCTCATT TTCTCTCTTA AATGCTCAAA TAAATGCAAA GCTCAGCACA TCTTTTCTAG	1998
TCACAAAAAT AATTCTTTTA TTTGCAGTTT ACGTATGATC TTAATTTCAA AACGATTCT	2058
TTGTTTTTGG CTTGATTTT CACAATGTTG CAAATATCAG GCTCCAGGG TTAATGTGG	2118
AATTGAAGTC TGCAGCCAGG CCTTGCAAAT TGAAGGTAAC TGGGGCAAAT GCCATTGAAA	2178
CCGCTAGTCT TATTTCCCTT CTACTTTTCT TTGGCACTCT TACTGCCTGT AAGGAGTAGA	2238
ACTGTTAAGG CACACTGTTG CTATACAGTT AACTCCCAT TCCATGTTTT GTCTTTCTTT	2298
TCCCATTTCT GGGGCTTACC TCCTGATACC TGCTTACTTT CTGGAAGTAG TGGGCAAGTA	2358
AGATTTGGCT CTTGGTTTCT GGAATTC	2385

(2) INFORMATION FOR SEQ ID NO:10:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 364 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Met Ala Ser Ser Ser Gly Ser Lys Ala Glu Phe Ile Val Gly Gly Lys
 1 5 10 15
 Tyr Lys Leu Val Arg Lys Ile Gly Ser Gly Ser Phe Gly Asp Ile Tyr
 20 25 30

-82-

Leu Ala Ile Asn Ile Thr Asn Gly Glu Glu Val Ala Val Lys Leu Glu
 35 40 45
 Ser Gln Lys Ala Arg His Pro Gln Leu Leu Tyr Glu Ser Lys Leu Tyr
 50 55 60
 Lys Ile Leu Gln Gly Gly Val Gly Ile Pro His Ile Arg Trp Tyr Gly
 65 70 75 80
 Gln Glu Lys Asp Tyr Asn Val Leu Val Met Asp Leu Leu Gly Pro Ser
 85 90 95
 Leu Glu Asp Leu Phe Asn Phe Cys Ser Arg Arg Phe Thr Met Lys Thr
 100 105 110
 Val Leu Met Leu Ala Asp Gln Met Ile Ser Arg Ile Glu Tyr Val His
 115 120 125
 Thr Lys Asn Phe Ile His Arg Asp Ile Lys Pro Asp Asn Phe Leu Met
 130 135 140
 Gly Ile Gly Arg His Cys Asn Lys Cys Leu Glu Ser Pro Val Gly Lys
 145 150 155 160
 Arg Lys Arg Ser Met Thr Val Ser Thr Ser Gln Asp Pro Ser Phe Ser
 165 170 175
 Gly Leu Asn Gln Leu Phe Leu Ile Asp Phe Gly Leu Ala Lys Lys Tyr
 180 185 190
 Arg Asp Asn Arg Thr Arg Gln His Ile Pro Tyr Arg Glu Asp Lys Asn
 195 200 205
 Leu Thr Gly Thr Ala Arg Tyr Ala Ser Ile Asn Ala His Leu Gly Ile
 210 215 220
 Glu Gln Ser Arg Arg Asp Asp Met Glu Ser Leu Gly Tyr Val Leu Met
 225 230 235 240
 Tyr Phe Asn Arg Thr Ser Leu Pro Trp Gln Gly Leu Lys Ala Ala Thr
 245 250 255
 Lys Lys Gln Lys Tyr Glu Lys Ile Ser Glu Lys Lys Met Ser Thr Pro
 260 265 270
 Val Glu Val Leu Cys Lys Gly Phe Pro Ala Glu Phe Ala Met Tyr Leu
 275 280 285
 Asn Tyr Cys Arg Gly Leu Arg Phe Glu Glu Ala Pro Asp Tyr Met Tyr
 290 295 300
 Leu Arg Gln Leu Phe Arg Ile Leu Phe Arg Thr Leu Asn His Gln Tyr
 305 310 315 320
 Asp Tyr Thr Phe Asp Trp Thr Met Leu Lys Gln Lys Ala Ala Gln Gln
 325 330 335
 Ala Ala Ser Ser Ser Gly Gln Gly Gln Gln Ala Gln Thr Pro Thr Gly
 340 345 350
 Lys Gln Thr Asp Lys Thr Lys Ser Asn Met Lys Gly
 355 360

-83-

(2) INFORMATION FOR SEQ ID NO:11:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 2914 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:

- (A) NAME/KEY: CDS
 (B) LOCATION: 265..1275

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

GAATTC	CCCGA	GAAACAAGTG	GCCCAGCCTG	GTAACCGCCG	AGAAGCCCTT	CACAACTGC	60
GGCCTG	GGCAA	AAAGAAACCT	GACTGAGCGG	CGGTGATCAG	GTTCCCCTCT	GCTGATTCTG	120
GGCCCCGAAC	CCCGGTAAAG	GCCTCCGTGT	TCCGTTTCCT	GCCGCCCTCC	TCCGTAGCCT		180
TGCCTAGTGT	AGGAGCCCCG	AGGCCTCCGT	CCTCTTCCCA	GAGGTGTCGG	GGCTTGGCCC		240
CAGCCTCCAT	CTTCGTCTCT	CAGG	ATG GCG AGT AGC AGC GGC TCC AAG GCT				291
			Met Ala Ser Ser Ser Gly Ser Lys Ala				
			1 5				
GAA TTC ATT GTC GGA GGG AAA TAT AAA CTG GTA CGG AAG ATC GGG TCT							339
Glu Phe Ile Val Gly Gly Lys Tyr Lys Leu Val Arg Lys Ile Gly Ser							
10 15 20 25							
GGC TCC TTC GGG GAC ATC TAT TTG GCG ATC AAC ATC ACC AAC GGC GAG							387
Gly Ser Phe Gly Asp Ile Tyr Leu Ala Ile Asn Ile Thr Asn Gly Glu							
30 35 40							
GAA GTG GCA GTG AAG CTA GAA TCT CAG AAG GCC AGG CAT CCC CAG TTG							435
Glu Val Ala Val Lys Leu Glu Ser Gln Lys Ala Arg His Pro Gln Leu							
45 50 55							
CTG TAC GAG AGC AAG CTC TAT AAG ATT CTT CAA GGT GGG GTT GGC ATC							483
Leu Tyr Glu Ser Lys Leu Tyr Lys Ile Leu Gln Gly Val Gly Ile							
60 65 70							
CCC CAC ATA CGG TGG TAT GGT CAG GAA AAA GAC TAC AAT GTA CTA GTC							531
Pro His Ile Arg Trp Tyr Gly Gln Glu Lys Asp Tyr Asn Val Leu Val							
75 80 85							
ATG GAT CTT CTG GGA CCT AGC CTC GAA GAC CTC TTC AAT TTC TGT TCA							579
Met Asp Leu Leu Gly Pro Ser Leu Glu Asp Leu Phe Asn Phe Cys Ser							
90 95 100 105							
AGA AGG TTC ACA ATG AAA ACT GTA CTT ATG TTA GCT GAC CAG ATG ATC							627
Arg Arg Phe Thr Met Lys Thr Val Leu Met Leu Ala Asp Gln Met Ile							
110 115 120							
AGT AGA ATT GAA TAT GTG CAT ACA AAG AAT TTT ATA CAC AGA GAC ATT							675
Ser Arg Ile Glu Tyr Val His Thr Lys Asn Phe Ile His Arg Asp Ile							
125 130 135							
AAA CCA GAT AAC TTC CTA ATG GGT ATT GGG CGT CAC TGT AAT AAG TTA							723
Lys Pro Asp Asn Phe Leu Met Gly Ile Gly Arg His Cys Asn Lys Leu							
140 145 150							

TTC	CTT	ATT	GAT	TTT	GGT	TTG	GCC	AAA	AAG	TAC	AGA	GAC	AAC	AGG	ACA
Phe	Leu	Ile	Asp	Phe	Gly	Leu	Ala	Lys	Lys	Tyr	Arg	Asp	Asn	Arg	Thr
155						160					165				
AGG	CAA	CAC	ATA	CCA	TAC	AGA	GAA	GAT	AAA	AAC	CTC	ACT	GGC	ACT	GCC
Arg	Gln	His	Ile	Pro	Tyr	Arg	Glu	Asp	Lys	Asn	Leu	Thr	Gly	Thr	Ala
170					175					180					185
CGA	TAT	GCT	AGC	ATC	AAT	GCA	CAT	CTT	GGT	ATT	GAG	CAG	AGT	CGC	CGA
Arg	Tyr	Ala	Ser	Ile	Asn	Ala	His	Leu	Gly	Ile	Glu	Gln	Ser	Arg	Arg
				190					195					200	
GAT	GAC	ATG	GAA	TCA	TTA	GGA	TAT	GTT	TTG	ATG	TAT	TTT	AAT	AGA	ACC
Asp	Asp	Met	Glu	Ser	Leu	Gly	Tyr	Val	Leu	Met	Tyr	Phe	Asn	Arg	Thr
			205					210					215		
AGC	CTG	CCA	TGG	CAA	GGG	CTA	AAG	GCT	GCA	ACA	AAG	AAA	CAA	AAA	TAT
Ser	Leu	Pro	Trp	Gln	Gly	Leu	Lys	Ala	Ala	Thr	Lys	Lys	Gln	Lys	Tyr
		220					225					230			
GAA	AAG	ATT	AGT	GAA	AAG	AAG	ATG	TCC	ACG	CCT	GTT	GAA	GTT	TTA	TGT
Glu	Lys	Ile	Ser	Glu	Lys	Lys	Met	Ser	Thr	Pro	Val	Glu	Val	Leu	Cys
	235					240					245				
AAG	GGG	TTT	CCT	GCA	GAA	TTT	GCG	ATG	TAC	TTA	AAC	TAT	TGT	CGT	GGG
Lys	Gly	Phe	Pro	Ala	Glu	Phe	Ala	Met	Tyr	Leu	Asn	Tyr	Cys	Arg	Gly
250					255					260					265
CTA	CGC	TTT	GAG	GAA	GCC	CCA	GAT	TAC	ATG	TAT	CTG	AGG	CAG	CTA	TTC
Leu	Arg	Phe	Glu	Glu	Ala	Pro	Asp	Tyr	Met	Tyr	Leu	Arg	Gln	Leu	Phe
				270					275					280	
CGC	ATT	CTT	TTC	AGG	ACC	CTG	AAC	CAT	CAA	TAT	GAC	TAC	ACA	TTT	GAT
Arg	Ile	Leu	Phe	Arg	Thr	Leu	Asn	His	Gln	Tyr	Asp	Tyr	Thr	Phe	Asp
			285					290					295		
TGG	ACA	ATG	TTA	AAG	CAG	AAA	GCA	GCA	CAG	CAG	GCA	GCC	TCT	TCC	AGT
Trp	Thr	Met	Leu	Lys	Gln	Lys	Ala	Ala	Gln	Gln	Ala	Ala	Ser	Ser	Ser
		300					305					310			
GGG	CAG	GGT	CAG	CAG	GCC	CAA	ACC	CCC	ACA	GGC	AAG	CAA	ACT	GAC	AAA
Gly	Gln	Gly	Gln	Gln	Ala	Gln	Thr	Pro	Thr	Gly	Lys	Gln	Thr	Asp	Lys
	315					320					325				
ACC	AAG	AGT	AAC	ATG	AAA	GGT	TTC	TAAGCATGAA	TTGAGGAACA	GAAGAAGCAG					
Thr	Lys	Ser	Asn	Met	Lys	Gly	Phe								
330					335										
AGCAGATGAT	CGGAGCAGCA	TTTGTTTCTC	CCCCAAATCTA	GAAATTTTTAG	TTCATATGTA										
1365															
CACTAGCCAG	TGGTTGTGGA	CAACCATTTA	CTTGGTGTA	AGAACTTAAT	TTCAGTATAA										
1425															
ACTGACTCTG	GGCAGCATTG	GTGATGCTGT	ATCCTGAGTT	GTAGCCTCTG	TAATTGTGAA										
1485															
TATTA ACTGA	GATAGTGAAA	CATGGTGTCC	GGTTTTCTAT	TGCATTTTTTT	CAAGTGGA										

-85-

AACTTAGTGA CTACTAGATT ATCCTTAGGA CTCTGCATTA ACTCTATAAT GTTCTTGGA 1965
 TTAAAAAAA AGCATATTTG TCACAGAAAT TTAGTTAACA TCTTACAACT GAACATGTAT 2025
 GTATGTTGCT TAGATAAATG TAATCACTGT AAACATCTAT ATGATCTGGG ATTTTGT TTTT 2085
 TATTTTGAAA TGGGAGCTTT TTTGTTTACA AGTTCATTAA AACTAAAAA CTGTTTCTGT 2145
 AAGGAAATGA GATTTTTTTT AAACAACAAA AAATGCCTTG CTGACTCACT ATTAAATAAA 2205
 AATCTCCCA ATTTTGTGAT AGACTACTTC AAGCCATTG TTACATGGTA TTCCTTGTCA 2265
 AGTCAATTTA GGTTCGTGT TATAACTTTT CCTCTTTTTT TAAGAAAAAT GAAAAAGTA 2325
 ATTCTTTTGT CTGAAGGGGA AAGGCATTCT TTCATTTTTT TCTTTTTTTT TTTTTTTTTT 2385
 TTATGACTTG CAGGCACAAT ATCTAGTACT GCAACTGCCA GAACTTGGTA TTGTAGCTGC 2445
 TGCCCGCTGA CTAGCAGCTG GACTGATTTT GAATAAAAAT GAAAGCAGTA CTGGGATTAC 2505
 AGGTGAGCCA CAGTGCCTGG CCCTTTTTTG TTTTATTGT CTGTCTCCCC ACTAGAAGGT 2565
 ACGCTCTACA AGGGCAGGGA TTTGTGCATC TTATTCATAG TGTTTCCAC GTGGCAGATG 2625
 CTCACTAAAG ATTTCAAAGG AGAACTGTG ATGGACTCGT TCTGTAGATG AGAGAACAGA 2685
 GGCACAGAGA CCTGTCCATG GTCCCTGGC AGAAGGAGGT GGGGTCTGGA TTCCACCCCA 2745
 GGGCTGCGTG GCTGCAGGAC CTCAGTGCTT GACTCCACAC TGCTGAGGGC TGTGAGTCCC 2805
 TGGCCAGCCC AGACACAGTC CTGCAGCCCA GGCTGAGCAT TCTCAGACCT TCATGGAGAT 2865
 GCCCACTCTC CTGTGAGCCT CTGCTTCCT TTGCCCAGGG CCGGAATTC 2914

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 337 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Met Ala Ser Ser Ser Gly Ser Lys Ala Glu Phe Ile Val Gly Gly Lys
 1 5 10 15
 Tyr Lys Leu Val Arg Lys Ile Gly Ser Gly Ser Phe Gly Asp Ile Tyr
 20 25 30
 Leu Ala Ile Asn Ile Thr Asn Gly Glu Glu Val Ala Val Lys Leu Glu
 35 40 45
 Ser Gln Lys Ala Arg His Pro Gln Leu Leu Tyr Glu Ser Lys Leu Tyr
 50 55 60
 Lys Ile Leu Gln Gly Gly Val Gly Ile Pro His Ile Arg Trp Tyr Gly
 65 70 75 80
 Gln Glu Lys Asp Tyr Asn Val Leu Val Met Asp Leu Leu Gly Pro Ser
 85 90 95
 Leu Glu Asp Leu Phe Asn Phe Cys Ser Arg Arg Phe Thr Met Lys Thr
 100 105 110

-86-

Val Leu Met Leu Ala Asp Gln Met Ile Ser Arg Ile Glu Tyr Val His
 115 120 125
 Thr Lys Asn Phe Ile His Arg Asp Ile Lys Pro Asp Asn Phe Leu Met
 130 135 140
 Gly Ile Gly Arg His Cys Asn Lys Leu Phe Leu Ile Asp Phe Gly Leu
 145 150 155 160
 Ala Lys Lys Tyr Arg Asp Asn Arg Thr Arg Gln His Ile Pro Tyr Arg
 165 170 175
 Glu Asp Lys Asn Leu Thr Gly Thr Ala Arg Tyr Ala Ser Ile Asn Ala
 180 185 190
 His Leu Gly Ile Glu Gln Ser Arg Arg Asp Asp Met Glu Ser Leu Gly
 195 200 205
 Tyr Val Leu Met Tyr Phe Asn Arg Thr Ser Leu Pro Trp Gln Gly Leu
 210 215 220
 Lys Ala Ala Thr Lys Lys Gln Lys Tyr Glu Lys Ile Ser Glu Lys Lys
 225 230 235 240
 Met Ser Thr Pro Val Glu Val Leu Cys Lys Gly Phe Pro Ala Glu Phe
 245 250 255
 Ala Met Tyr Leu Asn Tyr Cys Arg Gly Leu Arg Phe Glu Glu Ala Pro
 260 265 270
 Asp Tyr Met Tyr Leu Arg Gln Leu Phe Arg Ile Leu Phe Arg Thr Leu
 275 280 285
 Asn His Gln Tyr Asp Tyr Thr Phe Asp Trp Thr Met Leu Lys Gln Lys
 290 295 300
 Ala Ala Gln Gln Ala Ala Ser Ser Ser Gly Gln Gly Gln Gln Ala Gln
 305 310 315 320
 Thr Pro Thr Gly Lys Gln Thr Asp Lys Thr Lys Ser Asn Met Lys Gly
 325 330 335
 Phe

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 23 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vii) IMMEDIATE SOURCE:

- (B) CLONE: Protein Kinase

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..23
- (D) OTHER INFORMATION: /note= "Bases designated N at positions 3, 6, 9, 12 and 18 are Inosine."

-87-

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

GGNWSNGGNW SNTTYGGNGA YAT

23

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 23 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vii) IMMEDIATE SOURCE:

(B) CLONE: Protein Kinase

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..23
- (D) OTHER INFORMATION: /note= "Bases designated N at positions 6, 12 and 18 are Inosine."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

CAYGMNGAYA TNAARCCNGA YAA

23

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 24 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vii) IMMEDIATE SOURCE:

(B) CLONE: Protein Kinase

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..24
- (D) OTHER INFORMATION: /note= "Bases designated N at positions 7, 13 and 19 are Inosine."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

RTTRTCNGGY TTNATRTCNC KRTG

24

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 18 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vii) IMMEDIATE SOURCE:

(B) CLONE: Protein Kinase

-88-

- (ix) FEATURE:
 (A) NAME/KEY: CDS
 (B) LOCATION: 1..18
 (D) OTHER INFORMATION: /note= "Bases designated N at positions 1, 4, 7 and 13 are Inosine."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

NCCNARNSWY TCNARRTC

18

(2) INFORMATION FOR SEQ ID NO:17:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 20 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (vii) IMMEDIATE SOURCE:
 (B) CLONE: Protein Kinase

- (ix) FEATURE:
 (A) NAME/KEY: CDS
 (B) LOCATION: 1..20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

ATATAAACTG GTACGGAAGA

20

(2) INFORMATION FOR SEQ ID NO:18:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 17 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (vii) IMMEDIATE SOURCE:
 (B) CLONE: Protein Kinase

- (ix) FEATURE:
 (A) NAME/KEY: CDS
 (B) LOCATION: 1..17

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

ACATACGGTG GTATGGT

17

(2) INFORMATION FOR SEQ ID NO:19:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 19 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)

-89-

(vii) IMMEDIATE SOURCE:
(B) CLONE: Protein Kinase

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..19

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

ATGACATGGA ATCATTAGG

19

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vii) IMMEDIATE SOURCE:
(B) CLONE: Protein Kinase

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..19

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

CCTAATGATT CCATGTCAT

19

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vii) IMMEDIATE SOURCE:
(B) CLONE: Protein Kinase

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..18

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

TCAGGTACAT GTAATCCG

18

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 39 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

-90-

(vii) IMMEDIATE SOURCE:
(B) CLONE: Protein Kinase

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..39

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

CCTGATCGAT TCCAGCCTGA TCGCTACTTC TTCACCACT

39

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3627 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vii) IMMEDIATE SOURCE:
(B) CLONE: Protein Kinase

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1633..3204

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

GATCAGATGA TATAGCTTTT TGTGTGCCGT ACCTTTCGCG GATTCTGCCC GTATATCTTG	60
GTCCCTGAGC TATTTTCTGA GATTCTTTT GTTGCTTTGC CAAATCATTG GCGTCATTCA	120
TGGTCATACC AAATCCCAAT TTGGCAAAC TGGGTGTAA AGTATCTTGC TGTTCTTTTC	180
TAGTTGTGTC GAAGCTGTTT GAAGTGTCAT TAAAAAATC ATTGAATTCA TCAGGCTGGG	240
TATTAATATC ATCTATACTG TTATTATTGT TGCCTTTACT GTTATTCATA AATTGGGAAT	300
CGTAATCATT TGTCTAATTT TGGTGCTAGA AGACGAATTA GTGAACTCGT CCTCCTTTTC	360
TTGTTGAGCC TCTTTTTTAA ATTGATCAAA CAAGTCTTCT GCCTGTGATT TGTCGACTTT	420
CTTTGCGGTT AGTCTAGTGG GCTTTCTTGA CGAAGACAAA ATTGAATGTT TCTTTTTATC	480
TTGCGAGTTT AATACCGGTT TCTTTCTGCA TGCCGTTAAG ATGGAACCTCT CGTTTTAGTG	540
ACAGTGGTCT TGGGTGTGCT GCCTGTGGTG TTGTTTTTTG GGGCGAGAGA GCCTGTATTT	600
ACATTGAGTT TAGAACTGGA ATTGGAGCTT GGTTTTTGCC AATTAGAGAA AAAATCGTCA	660
ACACTATTTT CTTTGGAAGT CGACCTGGAA GCGTCTGAAT CGGTGTCCAA CGGTGAGTCC	720
GAAGAATCTT GACCGTTCAA GACTAATTCT GATGGGTATA ACTCCATATC CTTTTGAACC	780
TTCTTGTCGA GATGTATCTT ATATTTCTTA GCAACAGGGC TCGTATATTT TGTTTTCGCG	840
TCAACATTTG CTGTATTTAG TAGCTGTTTC CCATTGTTCT TTAAGAAAAA ATCACCAGCC	900
TTATGGTTCC CACCCAACCTT AAACCTTCTT AAATTGTTAA TTGTCCATTT ATCTAATGTA	960
GAAGACTTTA CAAAGGTGAT ATGAACACCC ATGTTTCTAT GCACAGCAGA GCATTGAATA	1020

-91-

CACAGCATCA CACCAAAAGG TACCGAAGTC CAGTAGGATT CTTGTTACCA CAATCAAAAC	1080
AAACTCGATT TTCCATGTTG CTACCTAGCT TCTGAAAAAC TTGTTGAGTA GTCTGTTCCG	1140
TGGCAAATGT TTCTCCTTCA TCGTTACTCA TTGTCGCTAT GTGTATACTA AATTGCTCAA	1200
GAAGACCGGA TCAACAAGTA CTTAACAAAT ACCCTTTCTT TGCTATCGCC TTGATCTCCT	1260
TTTATAAAAT GCCAGCTAAA TCGTGTTTAC GAAGAATAGT TGTTTTCTTT TTTTTTTTTT	1320
TTTTTCGAAA CTTTACCGTG TCGTCGAAAA TGACCAAACG ATGTTACTTT TCCTTTTGTG	1380
TCATAGATAA TACCAATATT GAAAGTAAAA TTTTAAACAT TCTATAGGTG AATTGAAAAG	1440
GGCAGCTTAG AGAGTAACAG GGGAACAGCA TTCGTAACAT CTAGGTACTG GTATTATTTG	1500
CTGTTTTTTTA AAAAAGAAGG AAATCCGTTT TGCAAGAATT GTCTGCTATT TAAGGGTATA	1560
CGTGCTACGG TCCACTAATC AAAAGTGGTA TCTCATTCTG AAGAAAAAGT GTAAAAAGGA	1620
CGATAAGGAA AG ATG TCC CAA CGA TCT TCA CAA CAC ATT GTA GGT ATT	1668
Met Ser Gln Arg Ser Ser Gln His Ile Val Gly Ile	
1 5 10	
CAT TAT GCT GTA GGA CCT AAG ATT GGC GAA GGG TCT TTC GGA GTA ATA	1716
His Tyr Ala Val Gly Pro Lys Ile Gly Glu Gly Ser Phe Gly Val Ile	
15 20 25	
TTT GAG GGA GAG AAC ATT CTT CAT TCT TGT CAA GCG CAG ACC GGT AGC	1764
Phe Glu Gly Glu Asn Ile Leu His Ser Cys Gln Ala Gln Thr Gly Ser	
30 35 40	
AAG AGG GAC TCT AGT ATA ATA ATG GCG AAC GAG CCA GTC GCA ATT AAA	1812
Lys Arg Asp Ser Ser Ile Ile Met Ala Asn Glu Pro Val Ala Ile Lys	
45 50 55 60	
TTC GAA CCG CGA CAT TCG GAC GCA CCC CAG TTG CGT GAC GAA TTT AGA	1860
Phe Glu Pro Arg His Ser Asp Ala Pro Gln Leu Arg Asp Glu Phe Arg	
65 70 75	
GCC TAT AGG ATA TTG AAT GGC TGC GTT GGA ATT CCC CAT GCT TAT TAT	1908
Ala Tyr Arg Ile Leu Asn Gly Cys Val Gly Ile Pro His Ala Tyr Tyr	
80 85 90	
TTT GGT CAA GAA GGT ATG CAC AAC ATC TTG ATT ATC GAT TTA CTA GAG	1956
Phe Gly Gln Glu Gly Met His Asn Ile Leu Ile Ile Asp Leu Leu Gly	
95 100 105	
CCA TCA TTG GAA GAT CTC TTT GAG TGG TGT GGT AGA AAA TTT TCA GTG	2004
Pro Ser Leu Glu Asp Leu Phe Glu Trp Cys Gly Arg Lys Phe Ser Val	
110 115 120	
AAA ACA ACC TGT ATG GTT GCC AAG CAA ATG ATT GAT AGA GTT AGA GCA	2052
Lys Thr Thr Cys Met Val Ala Lys Gln Met Ile Asp Arg Val Arg Ala	
125 130 135 140	
ATT CAT GAT CAC GAC TTA ATC TAT CGC GAT ATT AAA CCC GAT AAC TTT	2100
Ile His Asp His Asp Leu Ile Tyr Arg Asp Ile Lys Pro Asp Asn Phe	
145 150 155	
TTA ATT TCT CAA TAT CAA AGA ATT TCA CCT GAA GGA AAA GTC ATT AAA	2148
Leu Ile Ser Gln Tyr Gln Arg Ile Ser Pro Glu Gly Lys Val Ile Lys	
160 165 170	
TCA TGT GCC TCC TCT TCT AAT AAT GAT CCC AAT TTA ATA TAC ATG GTT	2196
Ser Cys Ala Ser Ser Ser Asn Asn Asp Pro Asn Leu Ile Tyr Met Val	
175 180 185	

-92-

GAC Asp 190	TTT Phe 190	GGT Gly 190	ATG Met 190	GCA Ala 190	AAA Lys 195	CAA Gln 195	TAT Tyr 195	AGA Arg 195	GAT Asp 195	CCA Pro 200	AGA Arg 200	ACG Thr 200	AAA Lys 200	CAA Gln 200	CAT His 200	2244
ATA Ile 205	CCA Pro 205	TAC Tyr 205	CGT Arg 205	GAA Glu 210	CGA Arg 210	AAA Lys 210	TCA Ser 210	TTG Leu 210	AGC Ser 215	GGT Gly 215	ACC Thr 215	GCC Ala 215	AGA Arg 215	TAT Tyr 220	ATG Met 220	2292
TCT Ser 225	ATT Ile 225	AAT Asn 225	ACT Thr 225	CAT His 225	TTT Phe 225	GGA Gly 230	AGA Arg 230	GAA Glu 230	CAG Gln 230	TCA Ser 230	CGT Arg 230	AGG Arg 235	GAT Asp 235	GAT Asp 235	TTA Leu 235	2340
GAA Glu 240	TCG Ser 240	CTA Leu 240	GGT Gly 240	CAC His 245	GTT Val 245	TTT Phe 245	TTT Phe 245	TAT Tyr 245	TTC Phe 245	TTG Leu 245	AGG Arg 250	GGA Gly 250	TCC Ser 250	TTG Leu 250	CCA Pro 250	2388
TGG Trp 255	CAA Gln 255	GGT Gly 255	TTG Leu 255	AAA Lys 260	GCA Ala 260	CCA Pro 260	AAC Asn 260	AAC Asn 260	AAA Lys 265	CTG Leu 265	AAG Lys 265	TAT Tyr 265	GAA Glu 265	AAG Lys 265	ATT Ile 265	2436
GGT Gly 270	ATG Met 270	ACT Thr 270	AAA Lys 275	CAG Gln 275	AAA Lys 275	TTG Leu 275	AAT Asn 275	CCT Pro 280	GAT Asp 280	GAT Asp 280	CTT Leu 280	TTA Leu 280	TTG Leu 280	AAT Asn 280	AAT Asn 280	2484
GCT Ala 285	ATT Ile 285	CCT Pro 290	TAT Tyr 290	CAG Gln 290	TTT Phe 290	GCC Ala 295	ACA Thr 295	TAT Tyr 295	TTA Leu 295	AAA Lys 295	TAT Tyr 300	GCA Ala 300	CGT Arg 300	TCC Ser 300	TTG Leu 300	2532
AAG Lys 305	TTC Phe 305	GAC Asp 305	GAA Glu 305	GAT Asp 305	CCG Pro 310	GAT Asp 310	TAT Tyr 310	GAC Asp 310	TAT Tyr 310	TTA Leu 315	ATC Ile 315	TCG Ser 315	TTA Leu 315	ATG Met 315	GAT Asp 315	2580
GAC Asp 320	GCT Ala 320	TTG Leu 320	AGA Arg 320	TTA Leu 325	AAC Asn 325	GAC Asp 325	TTA Leu 325	AAG Lys 325	GAT Asp 325	GAT Asp 330	GGA Gly 330	CAC His 330	TAT Tyr 330	GAC Asp 330	TGG Trp 330	2628
ATG Met 335	GAT Asp 335	TTG Leu 335	AAT Asn 335	GGT Gly 340	GGT Gly 340	AAA Lys 340	GGC Gly 340	TGG Trp 340	AAT Asn 345	ATC Ile 345	AAG Lys 345	ATT Ile 345	AAT Asn 345	AGA Arg 345	AGA Arg 345	2676
GCT Ala 350	AAC Asn 350	TTG Leu 350	CAT His 355	GGT Gly 355	TAC Tyr 355	GGA Gly 355	AAT Asn 360	CCA Pro 360	AAT Asn 360	CCA Pro 360	AGA Arg 360	GTC Val 360	AAT Asn 360	GGC Gly 360	AAT Asn 360	2724
ACT Thr 365	GCA Ala 365	AGA Arg 365	AAC Asn 365	AAT Asn 370	GTG Val 370	AAT Asn 370	ACG Thr 370	AAT Asn 375	TCA Ser 375	AAG Lys 375	ACA Thr 375	CGA Arg 375	AAT Asn 375	ACA Thr 375	ACG Thr 375	2772
CCA Pro 385	GTT Val 385	GCG Ala 385	ACA Thr 385	CCT Pro 385	AAG Lys 385	CAA Gln 390	CAA Gln 390	GCT Ala 390	CAA Gln 390	AAC Asn 390	AGT Ser 395	TAT Tyr 395	AAC Asn 395	AAG Lys 395	GAC Asp 395	2820
AAT Asn 400	TCG Ser 400	AAA Lys 400	TCC Ser 400	AGA Arg 405	ATT Ile 405	TCT Ser 405	TCG Ser 405	AAC Asn 405	CCG Pro 410	CAG Gln 410	AGC Ser 410	TTT Phe 410	ACT Thr 410	AAA Lys 410	CAA Gln 410	2868
CAA Gln 415	CAC His 415	GTC Val 415	TTG Leu 415	AAA Lys 420	AAA Lys 420	ATC Ile 420	GAA Glu 420	CCC Pro 425	AAT Asn 425	AGT Ser 425	AAA Lys 425	TAT Tyr 425	ATT Ile 425	CCT Pro 425	GAA Glu 425	2916
ACA Thr 430	CAT His 430	TCA Ser 430	AAT Asn 435	CTT Leu 435	CAA Gln 435	CGG Arg 435	CCA Pro 440	ATT Ile 440	AAA Lys 440	AGT Ser 440	CAA Gln 440	AGT Ser 440	CAA Gln 440	ACG Thr 440	TAC Tyr 440	2964
GAC Asp 445	TCC Ser 445	ATC Ile 445	AGT Ser 445	CAT His 450	ACA Thr 450	CAA Gln 450	AAT Asn 455	TCA Ser 455	CCA Pro 455	TTT Phe 455	GTA Val 455	CCA Pro 455	TAT Tyr 455	TCA Ser 455	AGT Ser 455	3012

-93-

TCT AAA GCT AAC CCT AAA AGA AGT AAT AAT GAG CAC AAC TTA CCA AAC	3060
Ser Lys Ala Asn Pro Lys Arg Ser Asn Asn Glu His Asn Leu Pro Asn	
465 470 475	
CAC TAC ACA AAC CTT GCA AAT AAG AAT ATC AAT TAT CAA AGT CAA CGA	3108
His Tyr Thr Asn Leu Ala Asn Lys Asn Ile Asn Tyr Gln Ser Gln Arg	
480 485 490	
AAT TAC GAA CAA GAA AAT GAT GCT TAT TCT GAT GAC GAG AAT GAT ACA	3156
Asn Tyr Glu Gln Glu Asn Asp Ala Tyr Ser Asp Asp Glu Asn Asp Thr	
495 500 505	
TTT TGT TCT AAA ATA TAC AAA TAT TGT TGT TGC TGT TTT TGT TGC TGT	3204
Phe Cys Ser Lys Ile Tyr Lys Tyr Cys Cys Cys Cys Phe Cys Cys Cys	
510 515 520	
TGATAAAGCG ATTTTATAC TTTTCTCTTT TTCCTTTTTT TTTTGTATTG GCTGTTTCCT	3264
TATGCCGCTC TTTCCCAATT TATGACTTTC CAATAATGTA TTATTTTGT TCTCTTCTC	3324
TCTGTTACCC TTTATTTTAT CATCTACAAT AATTGAATTC CGGAGAGGGT AAAGAAACAG	3384
GAAAAAGAAG AAAATGAGAC ATAGTCAGCA TCGTAATCGT TTCCTTCTG TATATTCCTT	3444
TATCAAAAGA CTACACGCAC ATATATATTA ATCCCGGTAT GTTTTGGTG TGCTAAATCT	3504
ATCTTCAAGC ACTATTATAG CATTTTTTTA AGAATATCCA AAATAATATG TAATTTATGA	3564
TTAATCAAGG TTCAAGAATT GGAGAAACCG TGAGCGACTT CTTTGATACT TGGATGTAAG	3624
CTT	3627

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 524 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

Met	Ser	Gln	Arg	Ser	Ser	Gln	His	Ile	Val	Gly	Ile	His	Tyr	Ala	Val
1				5					10					15	
Gly	Pro	Lys	Ile	Gly	Glu	Gly	Ser	Phe	Gly	Val	Ile	Phe	Glu	Gly	Glu
			20					25					30		
Asn	Ile	Leu	His	Ser	Cys	Gln	Ala	Gln	Thr	Gly	Ser	Lys	Arg	Asp	Ser
		35					40					45			
Ser	Ile	Ile	Met	Ala	Asn	Glu	Pro	Val	Ala	Ile	Lys	Phe	Glu	Pro	Arg
	50					55					60				
His	Ser	Asp	Ala	Pro	Gln	Leu	Arg	Asp	Glu	Phe	Arg	Ala	Tyr	Arg	Ile
	65				70					75					80
Leu	Asn	Gly	Cys	Val	Gly	Ile	Pro	His	Ala	Tyr	Tyr	Phe	Gly	Gln	Glu
			85						90					95	
Gly	Met	His	Asn	Ile	Leu	Ile	Ile	Asp	Leu	Leu	Gly	Pro	Ser	Leu	Glu
			100					105					110		
Asp	Leu	Phe	Glu	Trp	Cys	Gly	Arg	Lys	Phe	Ser	Val	Lys	Thr	Thr	Cys
		115					120					125			

-94-

Met Val Ala Lys Gln Met Ile Asp Arg Val Arg Ala Ile His Asp His
 130 135 140
 Asp Leu Ile Tyr Arg Asp Ile Lys Pro Asp Asn Phe Leu Ile Ser Gln
 145 150 155 160
 Tyr Gln Arg Ile Ser Pro Glu Gly Lys Val Ile Lys Ser Cys Ala Ser
 165 170 175
 Ser Ser Asn Asn Asp Pro Asn Leu Ile Tyr Met Val Asp Phe Gly Met
 180 185 190
 Ala Lys Gln Tyr Arg Asp Pro Arg Thr Lys Gln His Ile Pro Tyr Arg
 195 200 205
 Glu Arg Lys Ser Leu Ser Gly Thr Ala Arg Tyr Met Ser Ile Asn Thr
 210 215 220
 His Phe Gly Arg Glu Gln Ser Arg Arg Asp Asp Leu Glu Ser Leu Gly
 225 230 235 240
 His Val Phe Phe Tyr Phe Leu Arg Gly Ser Leu Pro Trp Gln Gly Leu
 245 250 255
 Lys Ala Pro Asn Asn Lys Leu Lys Tyr Glu Lys Ile Gly Met Thr Lys
 260 265 270
 Gln Lys Leu Asn Pro Asp Asp Leu Leu Leu Asn Asn Ala Ile Pro Tyr
 275 280 285
 Gln Phe Ala Thr Tyr Leu Lys Tyr Ala Arg Ser Leu Lys Phe Asp Glu
 290 295 300
 Asp Pro Asp Tyr Asp Tyr Leu Ile Ser Leu Met Asp Asp Ala Leu Arg
 305 310 315 320
 Leu Asn Asp Leu Lys Asp Asp Gly His Tyr Asp Trp Met Asp Leu Asn
 325 330 335
 Gly Gly Lys Gly Trp Asn Ile Lys Ile Asn Arg Arg Ala Asn Leu His
 340 345 350
 Gly Tyr Gly Asn Pro Asn Pro Arg Val Asn Gly Asn Thr Ala Arg Asn
 355 360 365
 Asn Val Asn Thr Asn Ser Lys Thr Arg Asn Thr Thr Pro Val Ala Thr
 370 375 380
 Pro Lys Gln Gln Ala Gln Asn Ser Tyr Asn Lys Asp Asn Ser Lys Ser
 385 390 395 400
 Arg Ile Ser Ser Asn Pro Gln Ser Phe Thr Lys Gln Gln His Val Leu
 405 410 415
 Lys Lys Ile Glu Pro Asn Ser Lys Tyr Ile Pro Glu Thr His Ser Asn
 420 425 430
 Leu Gln Arg Pro Ile Lys Ser Gln Ser Gln Thr Tyr Asp Ser Ile Ser
 435 440 445
 His Thr Gln Asn Ser Pro Phe Val Pro Tyr Ser Ser Ser Lys Ala Asn
 450 455 460
 Pro Lys Arg Ser Asn Asn Glu His Asn Leu Pro Asn His Tyr Thr Asn
 465 470 475 480

-95-

Leu Ala Asn Lys Asn Ile Asn Tyr Gln Ser Gln Arg Asn Tyr Glu Gln
485 490 495
Glu Asn Asp Ala Tyr Ser Asp Asp Glu Asn Asp Thr Phe Cys Ser Lys
500 505 510
Ile Tyr Lys Tyr Cys Cys Cys Cys Phe Cys Cys Cys
515 520

(2) INFORMATION FOR SEQ ID NO:25:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 6 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

- (vii) IMMEDIATE SOURCE:
 - (B) CLONE: Protein Kinase

- (ix) FEATURE:
 - (A) NAME/KEY: Peptide
 - (B) LOCATION: 1..6

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

Gly Pro Ser Leu Glu Asp
1 5

(2) INFORMATION FOR SEQ ID NO:26:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 9 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

- (vii) IMMEDIATE SOURCE:
 - (B) CLONE: Protein Kinase

- (ix) FEATURE:
 - (A) NAME/KEY: Peptide
 - (B) LOCATION: 1..9

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

Arg Asp Ile Lys Pro Asp Asn Phe Leu
1 5

(2) INFORMATION FOR SEQ ID NO:27:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 6 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

-96-

(vii) IMMEDIATE SOURCE:
(B) CLONE: Protein Kinase

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION: 1..6

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

His Ile Pro Tyr Arg Glu
1 5

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 6
(C) OTHER INFORMATION: /note= "The nucleotide at this position is inosine."

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 9
(C) OTHER INFORMATION: /note= "The nucleotide at this position is inosine."

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 12
(C) OTHER INFORMATION: /note= "The nucleotide at this position is inosine."

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 15
(C) OTHER INFORMATION: /note= "The nucleotide at this position is inosine."

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 21
(C) OTHER INFORMATION: /note= "The nucleotide at this position is inosine."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

GARYTNMGNY TNGGNAAYYT N

21

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

-97-

(ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 9

(C) OTHER INFORMATION: /note= "The nucleotide at this position is inosine."

(ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 12

(C) OTHER INFORMATION: /note= "The nucleotide at this position is inosine."

(ix) **FEATURE:**

(A) NAME/KEY: Modified-site

(B) LOCATION: 15

(C) OTHER INFORMATION: /note= "The nucleotide at this position is inosine."

(ix) **FEATURE:**

(A) NAME/KEY: Modified-site

(B) LOCATION: 18

(C) OTHER INFORMATION: /note= "The nucleotide at this position is inosine."

(ix) **FEATURE:**

(A) NAME/KEY: Modified-site

(B) LOCATION: 21

(C) OTHER INFORMATION: /note= "The nucleotide at this position is inosine."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

GTYTTRTTNC CNGGNCKNCC NAT

23

(2) INFORMATION FOR SEQ. ID NO:30:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 2405 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) **FEATURE:**

(A) NAME/KEY: CDS

(B) LOCATION: 67..1197

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

AAAGTGGAGT ACCGCAAAC T GATATGGAA AATAAAAAGA AAGACAAGGA CAAATCAGAT

60

GATAGA ATG GCA CGA CCT AGT GGT CGA TCG GGA CAC AAC ACT CGA GGA
Met Ala Arg Pro Ser Gly Arg Ser Gly His Asn Thr Arg Gly

108

Met Ala Arg Pro Ser Gly Arg Ser Gly His Asn Thr Arg Gly

1

5

10

-98-

ACT GGG TCT TCA TCG TCT GGA GTT TTA ATG GTT GGA CCT AAC TTT AGA Thr Gly Ser Ser Ser Ser Gly Val Leu Met Val Gly Pro Asn Phe Arg 15 20 25 30	156
GTT GGA AAA AAA ATT GGA TGT GGC AAT TTT GGA GAA TTA CGA TTA GGG Val Gly Lys Lys Ile Gly Cys Gly Asn Phe Gly Glu Leu Arg Leu Gly 35 40 45	204
AAA AAT TTA TAC ACA AAT GAA TAT GTG GCA ATT AAG TTG GAG CCC ATG Lys Asn Leu Tyr Thr Asn Glu Tyr Val Ala Ile Lys Leu Glu Pro Met 50 55 60	252
AAA TCA AGA GCA CCA CAG CTA CAT TTG GAA TAC AGA TTC TAT AAG CAG Lys Ser Arg Ala Pro Gln Leu His Leu Glu Tyr Arg Phe Tyr Lys Gln 65 70 75	300
TTA GGA TCT GGA GAT GGT ATA CCT CAA GTT TAC TAT TTC GGC CCC TGT Leu Gly Ser Gly Asp Gly Ile Pro Gln Val Tyr Tyr Phe Gly Pro Cys 80 85 90	348
GGT AAA TAC AAT GCT ATG GTG CTG GAA CTG CTG GGA CCT AGT TTG GAA Gly Lys Tyr Asn Ala Met Val Leu Glu Leu Leu Gly Pro Ser Leu Glu 95 100 105 110	396
GAC TTG TTT GAC TTG TGT GAC AGA ACA TTT TCT CTT AAA ACA GTT CTC Asp Leu Phe Asp Leu Cys Asp Arg Thr Phe Ser Leu Lys Thr Val Leu 115 120 125	444
ATG ATA GCT ATA CAA CTG ATT TCT CGC ATG GAA TAT GTC CAT TCA AAG Met Ile Ala Ile Gln Leu Ile Ser Arg Met Glu Tyr Val His Ser Lys 130 135 140	492
AAC TTG ATA TAC AGA GAT GTA AAA CCT GAG AAC TTC TTA ATA GGA CGA Asn Leu Ile Tyr Arg Asp Val Lys Pro Glu Asn Phe Leu Ile Gly Arg 145 150 155	540
CCA GGA AAC AAA ACC CAG CAA GTT ATT CAC ATT ATA GAT TTT GGT TTG Pro Gly Asn Lys Thr Gln Gln Val Ile His Ile Ile Asp Phe Gly Leu 160 165 170	588
GCA AAG GAA TAT ATT GAT CCG GAG ACA AAG AAA CAC ATA CCA TAC AGA Ala Lys Glu Tyr Ile Asp Pro Glu Thr Lys Lys His Ile Pro Tyr Arg 175 180 185 190	636
GAA CAC AAG AGC CTT ACA GGA ACA GCT AGA TAT ATG AGC ATA AAC ACA Glu His Lys Ser Leu Thr Gly Thr Ala Arg Tyr Met Ser Ile Asn Thr 195 200 205	684
CAT TTA GGA AAA GAA CAA AGT AGA AGA GAC GAT TTA GAA GCT TTA GGT His Leu Gly Lys Glu Gln Ser Arg Arg Asp Asp Leu Glu Ala Leu Gly 210 215 220	732
CAT ATG TTC ATG TAT TTT CTG AGA GGC AGT CTT CCT TGG CAA GGC TTA His Met Phe Met Tyr Phe Leu Arg Gly Ser Leu Pro Trp Gln Gly Leu 225 230 235	780
AAG GCT GAC ACA TTA AAG GAG AGG TAT CAG AAA ATT GGA GAT ACA AAA Lys Ala Asp Thr Leu Lys Glu Arg Tyr Gln Lys Ile Gly Asp Thr Lys 240 245 250	828
CGG GCT ACA CCA ATA GAA GTG TTA TGT GAA AAT TTT CCA GAA GAA ATG Arg Ala Thr Pro Ile Glu Val Leu Cys Glu Asn Phe Pro Glu Glu Met 255 260 265 270	876
GCA ACA TAT CTT CGT TAT GTA AGA AGG CTA GAT TTT TTT GAA AAA CCA Ala Thr Tyr Leu Arg Tyr Val Arg Arg Leu Asp Phe Phe Glu Lys Pro 275 280 285	924

-99-

GAC TAT GAC TAC TTA AGA AAG CTT TTT ACT GAC TTG TTT GAT CGA AAA Asp Tyr Asp Tyr Leu Arg Lys Leu Phe Thr Asp Leu Phe Asp Arg Lys 290 295 300	972
GGA TAT ATG TTT GAT TAT GAA TAT GAC TGG ATT GGT AAA CAG TTG CCT Gly Tyr Met Phe Asp Tyr Glu Tyr Asp Trp Ile Gly Lys Gln Leu Pro 305 310 315	1020
ACT CCA GTG GGT GCA GTT CAG CAA GAT CCT GCT CTG TCA TCA AAC AGA Thr Pro Val Gly Ala Val Gln Gln Asp Pro Ala Leu Ser Ser Asn Arg 320 325 330	1068
GAA GCA CAT CAA CAC AGA GAT AAG ATG CAA CAA TCC AAA AAC CAG GTT Glu Ala His Gln His Arg Asp Lys Met Gln Gln Ser Lys Asn Gln Val 335 340 345 350	1116
GTA AGT TCT ACA AAT GGA GAG TTA AAC ACA GAT GAC CCC ACC GCA GAC Val Ser Ser Thr Asn Gly Glu Leu Asn Thr Asp Asp Pro Thr Ala Asp 355 360 365	1164
GTT CAA ATG CAC CCA TCA CAG CCC CTA CTG AAG TAGAAGTGAT GGATGAAACC Val Gln Met His Pro Ser Gln Pro Leu Leu Lys 370 375	1217
AACTGCCAGA AAGTGGTGAA CATGTGGTGC TGCTGTTTTT TCAAACGAAG GAAAAGGAAA	1277
ACCATACAGC GCCACAAATG ACTCTGGACA CAGACAGATC CTGGGGAGTT ACTTACATGT	1337
TCATCTGCTG TCTTGTGATT AAAATCATCT CTGTAGTGAC CACGTATATT TTCAAGGACT	1397
CACTCTTAGA AACAAAAATG TCATACTTTC ATACTTCATT TTGTGGTTGT CTTACATTCT	1457
TTTTCTTTTT TTTTTTCTCT AATTTAACCT TTATGGAAGC TTTAAAGTTT TGTCAAAAAC	1517
ATGAGTGCTT TTGCCCCATC AGTGAATGGA ATGGACCAAT GAGGTGGTAT CAATGAATAT	1577
AGTTCCATAG AACATTTCCA GAAGTTCTTC TGTTGTAGAA AGCAGTACAG TATCTTAAGT	1637
GTCAACCAGT TATATACCTA ATCTGGTTTT TTATAACTTC TGTAAGAGCA TAATCAAACA	1697
GGAATTTTCT TTTCTCAGTG GATAATACAA CAGAGAAAAC AGAGTTGCCC AAATATTTAA	1757
AAGAAGTTAT TCCTTGAGAA GTTCATATTT TGTGACATCT GCATTGATTT CAGTATTACT	1817
GATGGTACTG TTATTCATAA GTCATATTAA CATTCTCTCC GTGAAATCAT GGTACAGTCG	1877
CTGCCCAGAG GTACTGAGGA AAAAGCAATA TGGGTTCGGC AGATGGTGGT GGTAAAATGA	1937
ATCTTAAGGA GTGTGGTAAA TATGCGTCCG CTTTTGTTGC ATCACTATGT GAAGTACTGT	1997
GTTGCAGAAG TGGCAAAAGC GCTTATTTTT AAAAATGCAA AATATTTGTA CAATGTA ACT	2057
TTATGCTTCC AAATAATAAT GTATGTTAGA CAGCAAGAAA TGAATACTTT AAAAAGTGAT	2117
GTATGTTGGA GTTATAAAGA AATACACTAA GGAGAGGTAG TAAATGTGAA CCTTGTTGCA	2177
GTGTATAAGG TGGAAGCCTA AAGAAATCTC ACCGAACTT ACTGCTGAAT GATTACATTC	2237
TCCCTTAAGC AGAAAACCTT GGATGTGCCA TGCAATGGTG TCTGTGTAAT TATTTTGCTC	2297
TTTGATTAAA AAAAAGACCC CCAGCAATAA AAAGTGGGTC ACTCTAAAAA AAAAAAAAAA	2357
AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA ACGACAGCAA CGGAATTC	2405

-100-

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 377 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

```

Met Ala Arg Pro Ser Gly Arg Ser Gly His Asn Thr Arg Gly Thr Gly
 1           5           10           15
Ser Ser Ser Ser Gly Val Leu Met Val Gly Pro Asn Phe Arg Val Gly
          20           25           30
Lys Lys Ile Gly Cys Gly Asn Phe Gly Glu Leu Arg Leu Gly Lys Asn
          35           40           45
Leu Tyr Thr Asn Glu Tyr Val Ala Ile Lys Leu Glu Pro Met Lys Ser
          50           55           60
Arg Ala Pro Gln Leu His Leu Glu Tyr Arg Phe Tyr Lys Gln Leu Gly
          65           70           75           80
Ser Gly Asp Gly Ile Pro Gln Val Tyr Tyr Phe Gly Pro Cys Gly Lys
          85           90           95
Tyr Asn Ala Met Val Leu Glu Leu Leu Gly Pro Ser Leu Glu Asp Leu
          100          105          110
Phe Asp Leu Cys Asp Arg Thr Phe Ser Leu Lys Thr Val Leu Met Ile
          115          120          125
Ala Ile Gln Leu Ile Ser Arg Met Glu Tyr Val His Ser Lys Asn Leu
          130          135          140
Ile Tyr Arg Asp Val Lys Pro Glu Asn Phe Leu Ile Gly Arg Pro Gly
          145          150          155          160
Asn Lys Thr Gln Gln Val Ile His Ile Ile Asp Phe Gly Leu Ala Lys
          165          170          175
Glu Tyr Ile Asp Pro Glu Thr Lys Lys His Ile Pro Tyr Arg Glu His
          180          185          190
Lys Ser Leu Thr Gly Thr Ala Arg Tyr Met Ser Ile Asn Thr His Leu
          195          200          205
Gly Lys Glu Gln Ser Arg Arg Asp Asp Leu Glu Ala Leu Gly His Met
          210          215          220
Phe Met Tyr Phe Leu Arg Gly Ser Leu Pro Trp Gln Gly Leu Lys Ala
          225          230          235          240
Asp Thr Leu Lys Glu Arg Tyr Gln Lys Ile Gly Asp Thr Lys Arg Ala
          245          250          255
Thr Pro Ile Glu Val Leu Cys Glu Asn Phe Pro Glu Glu Met Ala Thr
          260          265          270
Tyr Leu Arg Tyr Val Arg Arg Leu Asp Phe Phe Glu Lys Pro Asp Tyr
          275          280          285
Asp Tyr Leu Arg Lys Leu Phe Thr Asp Leu Phe Asp Arg Lys Gly Tyr
          290          295          300

```

-101-

Met Phe Asp Tyr Glu Tyr Asp Trp Ile Gly Lys Gln Leu Pro Thr Pro
 305 310 315 320

Val Gly Ala Val Gln Gln Asp Pro Ala Leu Ser Ser Asn Arg Glu Ala
 325 330 335

His Gln His Arg Asp Lys Met Gln Gln Ser Lys Asn Gln Val Val Ser
 340 345 350

Ser Thr Asn Gly Glu Leu Asn Thr Asp Asp Pro Thr Ala Asp Val Gln
 355 360 365

Met His Pro Ser Gln Pro Leu Leu Lys
 370 375

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1233 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..1041

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

AGA GTT GGA AAA AAA ATT GGA TGT GGC AAT TTT GGA GAA TTA CGA TTA	48
Arg Val Gly Lys Lys Ile Gly Cys Gly Asn Phe Gly Glu Leu Arg Leu	
1 5 10 15	
GGG AAA AAT TTA TAC ACA AAT GAA TAT GTG GCA ATT AAG TTG GAG CCC	96
Gly Lys Asn Leu Tyr Thr Asn Glu Tyr Val Ala Ile Lys Leu Glu Pro	
20 25 30	
ATG AAA TCA AGA GCA CCA CAG CTA CAT TTG GAA TAC AGA TTC TAT AAG	144
Met Lys Ser Arg Ala Pro Gln Leu His Leu Glu Tyr Arg Phe Tyr Lys	
35 40 45	
CAG TTA GGA TCT GGA GAT GGT ATA CCT CAA GTT TAC TAT TTC GGC CCT	192
Gln Leu Gly Ser Gly Asp Gly Ile Pro Gln Val Tyr Tyr Phe Gly Pro	
50 55 60	
TGT GGT AAA TAC AAT GCT ATG GTG CTG GAA CTG CTG GGA CCT AGT TTG	240
Cys Gly Lys Tyr Asn Ala Met Val Leu Glu Leu Leu Gly Pro Ser Leu	
65 70 75 80	
GAA GAC TTG TTT GAC TTG TGT GAC AGA ACA TTT TCT CTT AAA ACA GTT	288
Glu Asp Leu Phe Asp Leu Cys Asp Arg Thr Phe Ser Leu Lys Thr Val	
85 90 95	
CTC ATG ATA GCT ATA CAA CTG ATT TCT CGC ATG GAA TAT GTC CAT TCA	336
Leu Met Ile Ala Ile Gln Leu Ile Ser Arg Met Glu Tyr Val His Ser	
100 105 110	
AAG AAC TTG ATA TAC AGA GAT GTA AAA CCT GAG AAC TTC TTA ATA GGA	384
Lys Asn Leu Ile Tyr Arg Asp Val Lys Pro Glu Asn Phe Leu Ile Gly	
115 120 125	

-102-

CGA CCA GGA AAC AAA ACC CAG CAA GTT ATT CAC ATT ATA GAT TTT GGT Arg Pro Gly Asn Lys Thr Gln Gln Val Ile His Ile Ile Asp Phe Gly 130 135 140	432
TTG GCA AAG GAA TAT ATT GAT CCG GAG ACA AAG AAA CAC ATA CCA TAC Leu Ala Lys Glu Tyr Ile Asp Pro Glu Thr Lys Lys His Ile Pro Tyr 145 150 155 160	480
AGA GAA CAC AAG AGC CTT ACA GGA ACA GCT AGA TAT ATG AGC ATA AAC Arg Glu His Lys Ser Leu Thr Gly Thr Ala Arg Tyr Met Ser Ile Asn 165 170 175	528
ACA CAT TTA GGA AAA GAA CAA AGT AGA AGA GAC GAT TTA GAA GCT TTA Thr His Leu Gly Lys Glu Gln Ser Arg Arg Asp Asp Leu Glu Ala Leu 180 185 190	576
GGT CAT ATG TTC ATG TAT TTT CTG AGA GGC AGT CTT CCT TGG CAA GGC Gly His Met Phe Met Tyr Phe Leu Arg Gly Ser Leu Pro Trp Gln Gly 195 200 205	624
TTA AAG GTT GAC ACA TTA AAG GAG AGG TAT CAG AAA ATT GGA GAT ACA Leu Lys Val Asp Thr Leu Lys Glu Arg Tyr Gln Lys Ile Gly Asp Thr 210 215 220	672
AAA CGG GCT ACA CCA ATA GAA GTG TTA TGT GAA AAT TTT CCA GAA ATG Lys Arg Ala Thr Pro Ile Glu Val Leu Cys Glu Asn Phe Pro Glu Met 225 230 235 240	720
GCA ACA TAT CTT CGT TAT GTA AGA AGG CTA GAT TTT TTT GAA AAA CCA Ala Thr Tyr Leu Arg Tyr Val Arg Arg Leu Asp Phe Phe Glu Lys Pro 245 250 255	768
GAC TAT GAC TAC TTA AGA AAG CTT TTT ACT GAC TTG TTT GAT CGA AAA Asp Tyr Asp Tyr Leu Arg Lys Leu Phe Thr Asp Leu Phe Asp Arg Lys 260 265 270	816
GGA TAT ATG TTT GAT TAT GAA TAT GAC TGG ATT GGT AAA CAG TTG CCT Gly Tyr Met Phe Asp Tyr Glu Tyr Asp Trp Ile Gly Lys Gln Leu Pro 275 280 285	864
ACT CCA GTG GGT GCA GTT CAG CAA GAT CCT GCT CTG TCA TCA AAC AGA Thr Pro Val Gly Ala Val Gln Gln Asp Pro Ala Leu Ser Ser Asn Arg 290 295 300	912
GAA GCA CAT CAA CAC AGA GAT AAG ATG CAA CAA TCC AAA AAC CAG GTT Glu Ala His Gln His Arg Asp Lys Met Gln Gln Ser Lys Asn Gln Val 305 310 315 320	960
GTA AGT TCT ACA AAT GGA GAG TTA AAC ACA GAT GAC CCC ACC GCA GAC Val Ser Ser Thr Asn Gly Glu Leu Asn Thr Asp Asp Pro Thr Ala Asp 325 330 335	1008
GTT CAA ATG CAC CCA TCA CAG CCC CTA CTG AAG TAGAAGTGAT GGATGAAACC Val Gln Met His Pro Ser Gln Pro Leu Leu Lys 340 345	1061
AACTGCCAGA AAGTGTGAA CATGTGGTGC TGCTGTTTTT TCAAACGAAG GAAAAGGAAA	1121
ACCATACAGC GCCACAAATG ACTCTGGACA CAGACAGATC CTGGGGAGTT ACTTACATGT	1181
TCATCTGCTG TCTTGTGATT AAATCATCTC TGTAGTGACC ACGTATATTT TC	1233

-103-

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 347 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

```

Arg Val Gly Lys Lys Ile Gly Cys Gly Asn Phe Gly Glu Leu Arg Leu
 1             5             10             15
Gly Lys Asn Leu Tyr Thr Asn Glu Tyr Val Ala Ile Lys Leu Glu Pro
 20             25             30
Met Lys Ser Arg Ala Pro Gln Leu His Leu Glu Tyr Arg Phe Tyr Lys
 35             40             45
Gln Leu Gly Ser Gly Asp Gly Ile Pro Gln Val Tyr Tyr Phe Gly Pro
 50             55             60
Cys Gly Lys Tyr Asn Ala Met Val Leu Glu Leu Leu Gly Pro Ser Leu
 65             70             75             80
Glu Asp Leu Phe Asp Leu Cys Asp Arg Thr Phe Ser Leu Lys Thr Val
 85             90             95
Leu Met Ile Ala Ile Gln Leu Ile Ser Arg Met Glu Tyr Val His Ser
100            105            110
Lys Asn Leu Ile Tyr Arg Asp Val Lys Pro Glu Asn Phe Leu Ile Gly
115            120            125
Arg Pro Gly Asn Lys Thr Gln Gln Val Ile His Ile Ile Asp Phe Gly
130            135            140
Leu Ala Lys Glu Tyr Ile Asp Pro Glu Thr Lys Lys His Ile Pro Tyr
145            150            155            160
Arg Glu His Lys Ser Leu Thr Gly Thr Ala Arg Tyr Met Ser Ile Asn
165            170            175
Thr His Leu Gly Lys Glu Gln Ser Arg Arg Asp Asp Leu Glu Ala Leu
180            185            190
Gly His Met Phe Met Tyr Phe Leu Arg Gly Ser Leu Pro Trp Gln Gly
195            200            205
Leu Lys Val Asp Thr Leu Lys Glu Arg Tyr Gln Lys Ile Gly Asp Thr
210            215            220
Lys Arg Ala Thr Pro Ile Glu Val Leu Cys Glu Asn Phe Pro Glu Met
225            230            235            240
Ala Thr Tyr Leu Arg Tyr Val Arg Arg Leu Asp Phe Phe Glu Lys Pro
245            250            255
Asp Tyr Asp Tyr Leu Arg Lys Leu Phe Thr Asp Leu Phe Asp Arg Lys
260            265            270
Gly Tyr Met Phe Asp Tyr Glu Tyr Asp Trp Ile Gly Lys Gln Leu Pro
275            280            285
Thr Pro Val Gly Ala Val Gln Gln Asp Pro Ala Leu Ser Ser Asn Arg
290            295            300

```

Glu	Ala	His	Gln	His	Arg	Asp	Lys	Met	Gln	Gln	Ser	Lys	Asn	Gln	Val
305					310					315					320
Val	Ser	Ser	Thr	Asn	Gly	Glu	Leu	Asn	Thr	Asp	Asp	Pro	Thr	Ala	Asp
				325					330					335	
Val	Gln	Met	His	Pro	Ser	Gln	Pro	Leu	Leu	Lys					
			340					345							

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3505 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ix) FEATURE:

- (A) NAME/KEY: CDS
(B) LOCATION: 154..1398

GAATTC	CCGAC	AGGAAAGCGA	TGGTGAAAGC	GGGGCCGTGA	GGGGGGCGGA	GCCGGGAGCC	324	60
GGACCCG	CAG	TAGCGGCAGC	AGCGGCGCCG	CCTCCCGGAG	TTCAGACCCA	GGAAGCGGCC		120
GGGAGGG	CAG	GAGCGAATCG	GGCCGCCCGCC	GCC ATG GAG CTG AGA GTC GGG AAC				174
				Met Glu Leu Arg Val Gly Asn				
				1		5		
AGG TAC CGG CTG GGC CGG AAG ATC GGC AGC GGC TCC TTC GGA GAC ATC								222
Arg Tyr Arg Leu Gly Arg Lys Ile Gly Ser Gly Ser Phe Gly Asp Ile								
		10					15	20
TAT CTC GGT ACG GAC ATT GCT GCA GGA GAA GAG GTT GCC ATC AAG CTT								270
Tyr Leu Gly Thr Asp Ile Ala Ala Gly Glu Glu Val Ala Ile Lys Leu								
		25				30		35
GAA TGT GTC AAA ACC AAA CAC CCT CAG CTC CAC ATT GAG AGC AAA ATC								318
Glu Cys Val Lys Thr Lys His Pro Gln Leu His Ile Glu Ser Lys Ile								
		40			45		50	55
TAC AAG ATG ATG CAG GGA GGA GTG GGC ATC CCC ACC ATC AGA TGG TGC								366
Tyr Lys Met Met Gln Gly Gly Val Gly Ile Pro Thr Ile Arg Trp Cys								
				60			65	70
GGG GCA GAG GGG GAC TAC AAC GTC ATG GTG ATG GAG CTG CTG GGG CCA								414
Gly Ala Glu Gly Asp Tyr Asn Val Met Val Met Glu Leu Leu Gly Pro								
			75				80	85
AGC CTG GAG GAC CTC TTC AAC TTC TGC TCC AGG AAA TTC AGC CTC AAA								462
Ser Leu Glu Asp Leu Phe Asn Phe Cys Ser Arg Lys Phe Ser Leu Lys								
		90				95		100
ACC GTC CTG CTG CTT GCT GAC CAA ATG ATC AGT CGC ATC GAA TAC ATT								510
Thr Val Leu Leu Leu Ala Asp Gln Met Ile Ser Arg Ile Glu Tyr Ile								
		105			110			115
CAT TCA AAG AAC TTC ATC CAC CGG GAT GTG AAG CCA GAC AAC TTC CTC								558
His Ser Lys Asn Phe Ile His Arg Asp Val Lys Pro Asp Asn Phe Leu								
		120			125		130	135

-105-

ATG GGC CTG GGG AAG AAG GGC AAC CTG GTG TAC ATC ATC GAC TTC GGG Met Gly Leu Gly Lys Lys Gly Asn Leu Val Tyr Ile Ile Asp Phe Gly 140 145 150	606
CTG GCC AAG AAG TAC CGG GAT GCA CGC ACC CAC CAG CAC ATC CCC TAT Leu Ala Lys Lys Tyr Arg Asp Ala Arg Thr His Gln His Ile Pro Tyr 155 160 165	654
CGT GAG AAC AAG AAC CTC ACG GGG ACG GCG CGG TAC GCC TCC ATC AAC Arg Glu Asn Lys Asn Leu Thr Gly Thr Ala Arg Tyr Ala Ser Ile Asn 170 175 180	702
ACG CAC CTT GGA ATT GAA CAA TCC CGA AGA GAT GAC TTG GAG TCT CTG Thr His Leu Gly Ile Glu Gln Ser Arg Arg Asp Asp Leu Glu Ser Leu 185 190 195	750
GGC TAC GTG CTA ATG TAC TTC AAC CTG GGC TCT CTC CCC TGG CAG GGG Gly Tyr Val Leu Met Tyr Phe Asn Leu Gly Ser Leu Pro Trp Gln Gly 200 205 210 215	798
CTG AAG GCT GCC ACC AAG AGA CAG AAA TAC GAA AGG ATT AGC GAG AAG Leu Lys Ala Ala Thr Lys Arg Gln Lys Tyr Glu Arg Ile Ser Glu Lys 220 225 230	846
AAA ATG TCC ACC CCC ATC GAA GTG TTG TGT AAA GGC TAC CCT TCC GAA Lys Met Ser Thr Pro Ile Glu Val Leu Cys Lys Gly Tyr Pro Ser Glu 235 240 245	894
TTT GCC ACA TAC CTG AAT TTC TGC CGT TCC TTG CGT TTT GAC GAC AAG Phe Ala Thr Tyr Leu Asn Phe Cys Arg Ser Leu Arg Phe Asp Asp Lys 250 255 260	942
CCT GAC TAC TCG TAC CTG CGG CAG CTT TTC CGG AAT CTG TTC CAT CGC Pro Asp Tyr Ser Tyr Leu Arg Gln Leu Phe Arg Asn Leu Phe His Arg 265 270 275	990
CAG GGC TTC TCC TAT GAC TAC GTG TTC GAC TGG AAC ATG CTC AAA TTT Gln Gly Phe Ser Tyr Asp Tyr Val Phe Asp Trp Asn Met Leu Lys Phe 280 285 290 295	1038
GGT GCC AGC CGG GCC GCC GAT GAC GCC GAG CGG GAG CGC AGG GAC CGA Gly Ala Ser Arg Ala Ala Asp Asp Ala Glu Arg Glu Arg Arg Asp Arg 300 305 310	1086
GAG GAG CGG CTG AGA CAC TCG CGG AAC CCG GCT ACC CGC GGC CTC CCT Glu Glu Arg Leu Arg His Ser Arg Asn Pro Ala Thr Arg Gly Leu Pro 315 320 325	1134
TCC ACA GCC TCC GGC CGC CTG CGG GGG ACG CAG GAA GTG GCT CCC CCC Ser Thr Ala Ser Gly Arg Leu Arg Gly Thr Gln Glu Val Ala Pro Pro 330 335 340	1182
ACA CCC CTC ACC CCT ACC TCA CAC ACG GCT AAC ACC TCC CCC CGG CCC Thr Pro Leu Thr Pro Thr Ser His Thr Ala Asn Thr Ser Pro Arg Pro 345 350 355	1230
GTC TCC GGC ATG GAG AGA GAG CGG AAA GTG AGT ATG CGG CTG CAC CGC Val Ser Gly Met Glu Arg Glu Arg Lys Val Ser Met Arg Leu His Arg 360 365 370 375	1278
GGG GCC CCC GTC AAC ATC TCC TCG TCC GAC CTC ACA GGC CGA CAA GAT Gly Ala Pro Val Asn Ile Ser Ser Ser Asp Leu Thr Gly Arg Gln Asp 380 385 390	1326
ACC TCT CGC ATG TCC ACC TCA CAG ATT CCT GGT CGG GTG GCT TCC AGT Thr Ser Arg Met Ser Thr Ser Gln Ile Pro Gly Arg Val Ala Ser Ser 395 400 405	1374

-106-

GGT CTT CAG TCT GTC GTG CAC CGA	TGAGAACTCT CTTATTGCT GTGAAGGGCA	1428
Gly Leu Gln Ser Val Val His Arg		
410	415	
GACAATGCAT GGCTGATCTA CTCTGTTACC AATGGCTTTA CTAGTGACAC GTCCCCCGGT		1488
CTAGGATCGA AATGTTAACA CCGGGAGCTC TCCAGGCCAC TCACCCAGCG ACGCTCGTGG		1548
GGGAAACATA CTAAACGGAC AGACTCCAAG AGCTGCCACC GCTGGGGCTG CACTGCGGCC		1608
CCCCACGTGA ACTCGGTTGT AACGGGGCTG GGAAGAAAAG CAGAGAGAGA ATTGCAGAGA		1668
ATCAGACTCC TTTTCCAGGG CCTCAGCTCC CTCCAGTGGT GGCCGCCCTG TACTCCCTGA		1728
CGATTCCACT GTAAC TACCA ATCTTCTACT TGGTTAAGAC AGTTTTGTAT CATTTTGCTA		1788
AAAATTATTG GCTTAAATCT GTGTAAAGAA AATCTGTCTT TTTATTGTTT CTTGTCTGTT		1848
TTTGCGGTCT TACAAAAAAA ATGTTGACTA AGGAATTCTG AGACAGGCTG GCTTGGAGTT		1908
AGTGTATGAG GTGGAGTCGG GCAGGGAGAA GGTGCAGGTG GATCTCAAGG GTGTGTGCTG		1968
TGTTTGT TTT GCAGTGT TTT ATTGTCCGCT TTGGAGAGGA GATTTCTCAT CAAAAGTCCG		2028
TGGTGTGTGT GTGTGCCCGT GTGTGGTGGG ACCTCTTCAA CCTGATTTTG GCGTCTCACC		2088
CTCCCTCCTC CCGTAATTGA CATGCCTGCT GTCAGGAACT CTTGAGGCCC TCGGAGAGCA		2148
GTTAGGGACC GCAGGCTGCC GCGGGGCAGG GGTGCAGTGG GTGTTACCAG GCAAAGCACT		2208
GCGCGCTTCT TCCCCAGGAG GTGGGCAGGC AGCTGAGAGC TTGGAAGCAG AGGCTTTGAG		2268
ACCCTAGCAG GACAATTGGG AGTCCCAGGA TTCAAGGTGG AAGATGCGTT TCTGGTCCCT		2328
TGGGAGAGGA CTGTGAACCG AGAGGTGGTT ACTGTAGTGT TTGTTGCCTT GCTGCCTTTG		2388
CACTCAGTCC ATTTTCTCAG CACTCAATGC TCCTGTGCGG ATTGGCACTC CGTCTGTATG		2448
AATGCCTGTG GTTAAACCA GGAGCGGGGC TGTCCTTGCC ACGTGCCAAG ACTAGCTCAG		2508
AAAAGCCGGC AGGCCAGAAG GACCCACCCT GAGGTGCCAA GGAGCAGGTG ACTCTCCCAA		2568
CCGGACCCAG AACCTTCACG GCCAGAAAGT AGAGTCTGCG CTGTGACCTT CTGTTGGGCG		2628
CGTGTCTGTT GGTCAGAAGT GAAGCAGCGT GCGTGGGGCC GAGTCCCACC AGAAGGCAGG		2688
TGGCCTCCGT GAGCTGGTGC TGCCCCAGGC TCCATGCTGC TGTGCCCTGA GGTTCCCAGG		2748
ATGCCTTCTC GCCTCTCACT CCGCAGCACT TGGGCGGTAG CCAGTGGCCA TGTGCTCCCA		2808
ACCCCAATGC GCAGGGCAGT CTGTGTTCTG GGGCACTTCG GCTGGACCCC ATCACGATGG		2868
ACGATGTTCC CTTTGGA CTC TAGGGCTTCG AAGGTGTGCA CCTTGGTTCT CCCTTCTCCT		2928
CCCCAGAGTT CCCCCGATG CCATAACTGG CTGGCGTCCC AGAACACAGT TGTCAACCCC		2988
CCCACCAGCT GGCTGGCCGT CTGTCTGAGC CCATGGATGC TTTCTCAATC CTAGGCTGGT		3048
TACTGTGTAA GCGTGTGGA GTACGGCGCC TTGAGCGGGT GGGAGCTGTG TGTTGAAGTA		3108
CAGAGGGAGG TTGGGGTGGG TCAGAGCCGA GTTAAGAGAT TTTCTTTGTT GCTGGACCCC		3168
TTCTTGAAGG TAGACGTCCC CCACCCGGAG AGACGTCGCG CTGTGGCCTG AAGTGGCGCA		3228
AGCTTGCTTT GTAAATATCT GTGGTCCCGA TGAGTGCCCC AGAACGTTT TGCGAGGCAG		3288
CTCTGCGCCC GGGTTCAGC CCGAGCCTCG CCGGGTCGCG TCTTCGGAGT GCTTGTGACA		3348

-107-

GTCCTTGCCC AGTATCTAGT CCCCCTCGCC CCGTGCAGGA GACGTAGGTA GGACGTCGTG 3408
 TCAGCTGTGC ACTGACGGCC AGTCTCCGAG CTGTGCGTTT GTATCGCCAC TGTATTTGTG 3468
 TACTTTAACA ATCGTGTAAT TAATAAATTC GGAATTC 3505

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 415 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

Met Glu Leu Arg Val Gly Asn Arg Tyr Arg Leu Gly Arg Lys Ile Gly
 1 5 10 15
 Ser Gly Ser Phe Gly Asp Ile Tyr Leu Gly Thr Asp Ile Ala Ala Gly
 20 25 30
 Glu Glu Val Ala Ile Lys Leu Glu Cys Val Lys Thr Lys His Pro Gln
 35 40 45
 Leu His Ile Glu Ser Lys Ile Tyr Lys Met Met Gln Gly Gly Val Gly
 50 55 60
 Ile Pro Thr Ile Arg Trp Cys Gly Ala Glu Gly Asp Tyr Asn Val Met
 65 70 75 80
 Val Met Glu Leu Leu Gly Pro Ser Leu Glu Asp Leu Phe Asn Phe Cys
 85 90 95
 Ser Arg Lys Phe Ser Leu Lys Thr Val Leu Leu Leu Ala Asp Gln Met
 100 105 110
 Ile Ser Arg Ile Glu Tyr Ile His Ser Lys Asn Phe Ile His Arg Asp
 115 120 125
 Val Lys Pro Asp Asn Phe Leu Met Gly Leu Gly Lys Lys Gly Asn Leu
 130 135 140
 Val Tyr Ile Ile Asp Phe Gly Leu Ala Lys Lys Tyr Arg Asp Ala Arg
 145 150 155 160
 Thr His Gln His Ile Pro Tyr Arg Glu Asn Lys Asn Leu Thr Gly Thr
 165 170 175
 Ala Arg Tyr Ala Ser Ile Asn Thr His Leu Gly Ile Glu Gln Ser Arg
 180 185 190
 Arg Asp Asp Leu Glu Ser Leu Gly Tyr Val Leu Met Tyr Phe Asn Leu
 195 200 205
 Gly Ser Leu Pro Trp Gln Gly Leu Lys Ala Ala Thr Lys Arg Gln Lys
 210 215 220
 Tyr Glu Arg Ile Ser Glu Lys Lys Met Ser Thr Pro Ile Glu Val Leu
 225 230 235 240
 Cys Lys Gly Tyr Pro Ser Glu Phe Ala Thr Tyr Leu Asn Phe Cys Arg
 245 250 255

-108-

Ser Leu Arg Phe Asp Asp Lys Pro Asp Tyr Ser Tyr Leu Arg Gln Leu
 260 265 270

Phe Arg Asn Leu Phe His Arg Gln Gly Phe Ser Tyr Asp Tyr Val Phe
 275 280 285

Asp Trp Asn Met Leu Lys Phe Gly Ala Ser Arg Ala Ala Asp Asp Ala
 290 295 300

Glu Arg Glu Arg Arg Asp Arg Glu Glu Arg Leu Arg His Ser Arg Asn
 305 310 315 320

Pro Ala Thr Arg Gly Leu Pro Ser Thr Ala Ser Gly Arg Leu Arg Gly
 325 330 335

Thr Gln Glu Val Ala Pro Pro Thr Pro Leu Thr Pro Thr Ser His Thr
 340 345 350

Ala Asn Thr Ser Pro Arg Pro Val Ser Gly Met Glu Arg Glu Arg Lys
 355 360 365

Val Ser Met Arg Leu His Arg Gly Ala Pro Val Asn Ile Ser Ser Ser
 370 375 380

Asp Leu Thr Gly Arg Gln Asp Thr Ser Arg Met Ser Thr Ser Gln Ile
 385 390 395 400

Pro Gly Arg Val Ala Ser Ser Gly Leu Gln Ser Val Val His Arg
 405 410 415

(2) INFORMATION FOR SEQ ID NO:36:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 40 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

CTAGATCTAG CTAGACCATG GTAGTTTTTT CTCCTTGACG

40

(2) INFORMATION FOR SEQ ID NO:37:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 21 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

CATGCCATGG CACGACCTAG T

21

-109-

(2) INFORMATION FOR SEQ ID NO:38:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 40 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

CTAGATCTAG CTAGACCATG GTAGTTTTTT CTCCTTGACG

40

(2) INFORMATION FOR SEQ ID NO:39:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 38 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

GAATCGGGCC GCCGAGATCT CATATGGAGC TGAGAGTC

38

(2) INFORMATION FOR SEQ ID NO:40:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 21 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

CCCGGATCTA GCAGATCTCA T

21

(2) INFORMATION FOR SEQ ID NO:41:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 15 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

Ala Ser Ser Ser Gly Ser Lys Ala Glu Phe Ile Val Gly Gly Tyr
1 5 10 15

-110-

(2) INFORMATION FOR SEQ ID NO:42:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

Arg Ser Met Thr Val Ser Thr Ser Gln Asp Pro Ser Phe Ser Gly Tyr
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:43:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

TACATCTAGA ATTATGGCGA GTAGCAGCGG C

31

(2) INFORMATION FOR SEQ ID NO:44:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

AATGGATCCT TAGAAACCTG TGGGGGT

27

(2) INFORMATION FOR SEQ ID NO:45:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 36 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

AATGGATCCT TAGAAACCTT TCATGTTACT CTTGGT

36

-111-

(2) INFORMATION FOR SEQ ID NO:46:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 31 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:

TACATCTAGA ATTATGGAGC TGAGAGTCGG G

31

(2) INFORMATION FOR SEQ ID NO:47:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 27 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:

GGATCCTCAT CGGTGCACGA CAGACTG

27

(2) INFORMATION FOR SEQ ID NO:48:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 37 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:

TACATCTAGA ATTATGGCAC GACCTAGTGG TCGATCG

37

(2) INFORMATION FOR SEQ ID NO:49:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 25 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:

GGGGATCCTA CTTAGTAGG GGCTG

25

-112-

(2) INFORMATION FOR SEQ ID NO:50:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:50:

Arg Ser Gly His Asn Thr Arg Gly Thr Gly Ser Ser
1 5 10

(2) INFORMATION FOR SEQ ID NO:51:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:51:

Arg Leu Gly His Asn Thr Arg Gly Thr Gly Ser Ser
1 5 10

(2) INFORMATION FOR SEQ ID NO:52:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:52:

Ser Ser Arg Pro Lys Thr Asp Val Leu Val Gly
1 5 10

(2) INFORMATION FOR SEQ ID NO:53:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:53:

Lys Ser Asp Asn Thr Lys Ser Glu Met Lys His Ser
1 5 10

-113-

(2) INFORMATION FOR SEQ ID NO:54:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:54:

Gly Thr Asp Ile Ala Ala Gly Glu
1 5

(2) INFORMATION FOR SEQ ID NO:55:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:55:

Glu Arg Arg Asp Arg Glu Glu Arg Leu Arg
1 5 10

(2) INFORMATION FOR SEQ ID NO:56:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:56:

Thr Gly Lys Gln Thr Asp Lys Thr Lys Ser Asn Met Lys Gly Tyr
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:57:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:57:

Asp Leu Leu Gly Pro Ser Leu Glu Asp Leu Phe Gly Tyr
1 5 10

-114-

CLAIMS

1. An isolated polynucleotide sequence encoding a polypeptide with an amino acid sequence having at least about 35% homology in the protein kinase domain with the polynucleotide encoding *HRR25* protein kinase.
- 5 2. The polynucleotide of claim 1 wherein the encoded polypeptide possesses casein kinase activity.
3. The polynucleotide of claim 1 wherein the encoded polypeptide possesses protein-serine/threonine kinase activity.
4. The polynucleotide of claim 1 wherein the encoded polypeptide
10 possesses protein-tyrosine kinase activity.
5. The polynucleotide of claim 1 wherein the encoded polypeptide possess protein-serine/threonine and protein-tyrosine kinase activity.
6. The polynucleotide of claim 1, wherein the polypeptide is characterized as:
15 a) *promoting normal meiotic recombination; and*
 b) *promoting the repair a DNA strand break which occurs at the cleavage site:*

↓
 CAACAG
20 GTTGTC
 ↑
7. The polynucleotide of claim 1, wherein the polypeptide comprises an amino acid sequence selected from the group consisting of GPSLED (amino acids 86 to 91 in SEQ ID NO: 2), RDIKPDNFL (amino acids 127 to 135
25 in SEQ ID NO: 2), HIPYRE (amino acids 164 to 169 in SEQ ID NO: 2), and

-115-

SVN (amio acids 181 to 183 in SEQ ID NO: 2) and conservative variations thereof.

8. The polynucleotide of claim 1, selected from the group consisting of RNA, mRNA, genomic DNA and cDNA.

5 9. An antisense polynucleotide according to claim 1.

10. The polynucleotide of claim 1, selected from the group consisting of the DNA sequences of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 23, 30, 31, and 34.

10 11. An isolated and purified polypeptide encoded by a DNA sequence of claim 10.

12. The polynucleotide of claim 1 wherein the polynucleotide is isolated from organisms selected from the group consisting of *Saccharomyces*, *Schizosaccharomyces*, human, bovine, porcine, murine, avian and *Drosophila* species.

15 13. An autonomously replicating DNA vector comprising a DNA according to claim 8.

14. A procaryotic or eukaryotic host cell stably transformed or transfected with a DNA according to claim 8.

20 15. A method for the production of a polypeptide possessing protein kinase and/or recombination/repair promoting activity comprising growing a host cell according to claim 14 in a suitable nutrient medium and isolating the desired polypeptide from said host cell or from the medium of its growth.

16. A polypeptide product of the method of claim 15.

-116-

17. An antibody substance specific for a polypeptide of claim 15.
18. A monoclonal antibody according to claim 17.
19. A method for identifying a composition which modulates the protein kinase and/or recombination/repair promoting activity of an HRR25-like protein comprising:
- 5
- (a) incubating a system of components comprising the composition and the protein in the presence of a substrate for said protein wherein incubation is carried out under conditions sufficient to allow the components to interact; and
- 10 (b) measuring the change in activity of said protein on said substrate.
20. The method of claim 19 wherein the activity is promotion of repair of a DNA double strand break.
21. The method of claim 19 wherein the activity is protein kinase activity.
- 15 22. A method of treating a cell proliferative disorder associated with an HRR25-like protein comprising administering, to a subject with the disorder, a therapeutically effective amount of a composition which modulates the activity of the protein.

A.

HRR25	1/	MDLRVGRKERIGR	KIG	GSFG	IM	GTNII	---	SGEVA	TK	PS	IHS	---	RHPQLD	YS	SH	RY	ISG
CDC28	1/	MSGELANYKRLEK	VG	GT	YV	YK	AL	LP	GG	QR	VA	---	IL	SE	DE	GP	STAIR
K991	1/	MARTITEDIP	SQYK	LV	DI	IG	---	GAY	CV	CS	A	---	SG	IN	VA	IK	QPI
RAF1	346/	SEVM	LS	TR	IG	---	SE	FG	Y	TK	WH	---	---	VA	KL	YV	DP

HRR25	62/	Q	M	P	F	R	H	E	C	E	G	---	E	Y	N	A	M	I
CDC28	66/	D	N	I	V	R	L	Y	D	I	V	---	H	K	L	Y	M	F
K991	68/	H	E	N	I	S	I	L	D	K	V	---	V	S	I	D	K	
RAF1	401/	I	M	I	L	F	M	G	Y	M	T	---	D	N	I	M	Q	

HRR25	123/	S	F	I	R	D	I	N	P	A	N	---	G	V	G	I	R
CDC28	116/	R	I	L	H	R	D	L	P	Q	N	---	N	L	K	I	Q
K991	122/	Q	V	H	R	D	I	N	P	S	N	---	D	L	K	I	Q
RAF1	461/	N	I	H	R	D	M	K	S	N	I	---	T	V	K	I	Q

HRR25	190/	S	I	R	D	I	N	P	A	N	---	G	V	G	I	R	
CDC28	190/	S	I	R	D	I	N	P	A	N	---	G	V	G	I	R	
K991	203/	T	T	A	P	D	I	N	P	A	N	---	G	V	G	I	
RAF1	524/	S	F	I	R	D	I	N	P	A	N	---	G	V	G	I	

HRR25	243/	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
CDC28	257/	L	S	Q	V	V	P	S	---	---	---	---	---	---	---	---
K991	276/	H	E	T	V	H	S	K	T	---	---	---	---	---	---	---
RAF1	574/	---	---	---	---	---	---	---	---	---	---	---	---	---	---	

HRR25	288/	L	I	E	K	G	D	L	N	A	S	A	S	N	S	T
HRR25	361/	P	E	E	L	N	A	D	K	G	M	E	N	L	R	Q
HRR25	451/	R	A	T	Q	Y	P	P	Q	I	N	S	N	E	N	



FIGURE 1

```

Hrr25      -----MDLRVGRKFRIGRKIGSGSFGDIYHGNTNLISG-----EEVA
Yck1/cki2  ----SSRDDSTIIGLHYKIGKKIGEGSFGVLFEGTNMING-----VPVA
Yck2/cki1  -SGSQSRDDSTIIGLHYKIGKKIGEGSFGVLFEGTNMING-----LPVA
Nuf1       ---MSQRSSQHIYGIHYAVGPKIGEGSFGVIFEGENILHSCQAQTGSKRDSSIIMANEPVA
Hhp1       -----MALDLRIGNKYRIGRKIGSGSFGDIYLGTVVSG-----EEVA
Hhp2       -----MTVVVDIKIGNKYRIGRKIGSGSFGQIYLGNTVNG-----EQVA
CKIa1Hu    MASSSGSKAEFIVGGKRYKLVRKIGSGSFGDIYLAINITNG-----EEVA
CKIa2Hu    MASSSGSKAEFIVGGKRYKLVRKIGSGSFGDIYLAINITNG-----EEVA
CKIa3Hu    MASSSGSKAEFIVGGKRYKLVRKIGSGSFGDIYLAINITNG-----EEVA
Common     -----G-KYKIGRKIGSGSFGDIY-GTN--NG-----E-VA

Hrr25      IKLESIRSRHPQLDYESRVYRILSGGVGIPFIRWFGREGEYNAMVIDLLGPSLEDLFNYCH
Yck1/cki2  IKFEPRKTEAPQLRDEYKTYKILNGTPNIPYAYYFGQEGLENILVIDLLGPSLEDLFDWCG
Yck2/cki1  IKFEPRKTEAPQLKDEYRTYKILAGTPGIPQEYYFGQEGLENILVIDLLGPSLEDLFDWCG
Nuf1       IKFEPRHSDAPQLRDEFRAYRILNGCVGIPHAYYFGQEGMHNILIIDLLGPSLEDLFEWCN
Hhp1       IKLESTRARHPQLEYEYRVYRILSGGVGIPFVRWFGVECDYNAMVMDLLGPSLEDLNFNCG
Hhp2       VKLEPLKARHHQLEYEYFRVYNILKGNIGIPTIRWFGVTNSYNAMVMDLLGPSLEDLFCYCG
CKIa1Hu    VKLESQKARHPQLLYESKLYKILQGGVGIPHIRWYGQEKDYNVLMMDLLGPSLEDLNFNCS
CKIa2Hu    VKLESQKARHPQLLYESKLYKILQGGVGIPHIRWYGQEKDYNVLMMDLLGPSLEDLNFNCS
CKIa3Hu    VKLESQKARHPQLLYESKLYKILQGGVGIPHIRWYGQEKDYNVLMMDLLGPSLEDLNFNCS
Common     IKLEP-KA-HPQL-YE-RVYKIL-G-VGIP--RWFG--G-YNALVIDLLGPSLEDLF--CG

Hrr25      RRFSFKTVIMLALQMFECRIQYIHGRSFIHRDIKPDNFLMG--VGRRGST-----
Yck1/cki2  RKFSVKTVVQVAVQMILTIEDLHAHDLIYRDIKPDNFLIGRPGQPDANN-----
Yck2/cki1  RRFSVKTVLLADQLITLIEDLHAHDLIYRDIKPDNFLIGRPGQPDANK-----
Nuf1       RKFSVKTTTCMVAKQMIDRVRAIHHDLIYRDIKPDNFLISQYQRISPEGKVIKSCASSNN
Hhp1       RKFSLKTVLLADQLISRIEFIHKSFLHRDIKPDNFLMG--IGKRGNO-----
Hhp2       RKFTLKTVLLADQLISRIEYVHKSFLHRDIKPDNFLM--KKHSNV-----
CKIa1Hu    RRFTMKTVLMADQMISRIEYVHTKNFIHRDIKPDNFLMG--IGRHCKN-----
CKIa2Hu    RRFTMKTVLMADQMISRIEYVHTKNFIHRDIKPDNFLMG--IGRHCKN-----
CKIa3Hu    RRFTMKTVLMADQMISRIEYVHTKNFIHRDIKPDNFLMG--IGRHCKNCLESVPGKRKRS
Common     RRFS-KTVLMADQMISRIEYIH--DFIHRDIKPDNFLMG--G--N-----

Hrr25      -----VHVIDFGLSKKYRDFNTHRHIPYRENKSLTGTARYASVNTHLGIE
Yck1/cki2  -----IHLIDFGMAKQYRDPKTKQHIPPYREKKSLSGTARYMSINTHIGRE
Yck2/cki1  -----VHLIDFGMAKQYRDPKTKQHIPPYREKKSLSGTARYMSINTHIGRE
Nuf1       NDPNL-----IYMVDFGMAKQYRDPRTKQHIPPYRERKKSLSGTARYMSINTHFGRE
Hhp1       -----VNIIDFGLAKKYRDHKTHLHIPYRENKNLTGTARYASINTHLGIE
Hhp2       -----VTMIDFGLAKKYRDFKTHVHIPYRDKNLTGTARYASINTHIGIE
CKIa1Hu    -----LFLIDFGLAKKYRDNRTQHIPYREDKNLTGTARYASINAHLGIE
CKIa2Hu    -----LFLIDFGLAKKYRDNRTQHIPYREDKNLTGTARYASINAHLGIE
CKIa3Hu    MTVSTSQDPSFSGLNQLFLIDFGLAKKYRDNRTQHIPYREDKNLTGTARYASINAHLGIE
Common     -----VHLIDFGLAKKYRDPKTKQHIPPYRENKSLTGTARYASINTHLGIE

```

FIGURE 2A


```

Hrr25      QSRDDLES LGYVLIYFCRGS LPWQGLKATTKKQKYDRIMEKKLNVSVETLCSGLPL--EF
Yck1/cki2  QSRDDMEALGHVFFYFLRGHLPWQGLQAPNNKQKYEKIGEEKRLTNLYDLAQGLPV--QF
Yck2/cki1  QSRDDMEAMGHVFFYFLRGQLPWQGLQAPNNKQKYEKIGEEKRLTNLYDLAQGLPI--QF
Nuf1       QSRDDLES LGHVFFYFLRGSLPWQGLKAPNNKLYEKIGMTKQKLNPDLLNNAIPYQF
Hhp1       QSRDDLES LGYVLVYFCRGS LPWQGLAATTKKQKYEKIMEKKISTPTEVLCRGFPQ--EF
Hhp2       QSRDDLES LGYVLLYFCRGS LPWQGLQADTKQKYQRIRDTKIGTPLEVLCRGLPE--EF
CKIa1Hu    QSRDDMES LGYVLMYFNRTSLPWQGLKAATTKKQKYEKISEKKMSTPVEVLCRGFPA--EF
CKIa2Hu    QSRDDMES LGYVLMYFNRTSLPWQGLKAATTKKQKYEKISEKKMSTPVEVLCRGFPA--EF
CKIa3Hu    QSRDDMES LGYVLMYFNRTSLPWQGLKAATTKKQKYEKISEKKMSTPVEVLCRGFPA--EF
Common     QSRDDMES LGYVL-YF-RGSLPWQGLKAPTCKKQKYEKIGEEK--T-LEVLC-GLP---EF

Hrr25      -QEYMA YCKNLKFDEKPDYLF LARLFKDLS IKLEYHNDHLFDWTMLRYTKAMVE
Yck1/cki2  GRYLEI VERSLSFE ECPDYEGYRKL LLSVLDDLGETADGQYDWMKLN DGRG
Yck2/cki1  GRYLEI VERNLSFE ETPDYEGYRML LLSVLDDLGETADGQYDWMKLN GGRG
Nuf1       -ATY LKYARSLKFDEDPDYDYLISLMD DALRLNDLKDDGHYDWM DLNGGKG
Hhp1       -SIY LNYTRSLRFDDKPDAYFRKRLRKDFCRQSEEFNYMLFDWTLKRKT
Hhp2       -T-YMCYTRQLSFTEKPNYAYLMKAFRDLLIRKGYQYDYVFDWMILK
CKIa1Hu    -AMY LNYCRGLRFEEAPDYMYLRQLFRILFRTL NHQYDYTFDWTMLKQKAAQQAASSSGQG
CKIa2Hu    -AMY LNYCRGLRFEEAPDYMYLRQLFRILFRTL NHQYDYTFDWTMLKQKAAQQAASSSGQG
CKIa3Hu    -AMY LNYCRGLRFEEAPDYMYLRQLFRILFRTL NHQYDYTFDWTMLKQKAAQQAASSSGQG
Common     ---YL-Y-R-LSFDEKPDY-YLR-LF--LL-----D--FDWT-L-

CKIa1Hu    QQAQTPTGTF
CKIa2Hu    QQAQTPTGTFKQTDKTKSNMKG
CKIa3Hu    QQAQTPTGTFKQTDKTKSNMKG

```

FIGURE 2B

[illegible]